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THE ACTION OF SYNTHETIC QUINOLINE COMPOUNDS ON AVIAN MALARIA.

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I. INTRODUCTION AND HISTORICAL.

THE present paper embodies some of the results obtained in the course of work carried on for some years on the chemotherapy of avian malaria on behalf of the Chemotherapy Committee of the Medical Research Council, London. This work involved the testing of numerous alkaloids and new synthetic compounds, especially of the quinoline series, for action on bird malaria.

Many workers have previously investigated the chemotherapy of avian malaria, and amongst the most important of them are the following: Kopanaris (1911) found that quinine kills the parasites of avian malaria when allowed to act on them *in vitro*; and that when given intramuscularly it has a definite therapeutic action on infected birds. Kopanaris also found that the gametes are more resistant to the action of quinine than are the schizonts, and that it has no prophylactic action against blood-inoculated malaria of birds. Et. and Ed. Sergent (1921) further investigated the action of quinine and showed that large doses during the acute stage cause the disappearance of parasites from the peripheral blood within 48 hours, and that prolonged small doses, daily or on alternate days, after inoculation prevent acute infection during treatment but acute attacks may supervene after the cessation of treatment. The drug was given subcutaneously. Boyd (1926) investigated the action of quinine and fourteen synthetic quinoline compounds when administered orally

and intramuscularly, and found that quinine is the most effective. Giemsa, Weise and Tropp (1926) tested twenty-five quinine derivatives by subcutaneous injection, and found that there is a parallelism between the action of the effective compounds on avian and human malaria. Further investigations on the action of quinine and cinchona alkaloids were made by Goodson, Henry and Macfie (1930).

Roehl (1926) was the first to describe the action of a new synthetic compound, the anti-malarial properties of which were revealed by tests on birds and which was then found to be of value against human malaria. This substance, plasmoquine, Roehl found to be very effective against malaria of birds, and when given orally it has a therapeutic index $\left[\frac{\text{Maximum tolerated dose}}{\text{Minimum effective dose}}\right]$ of 1 : 30, whereas quinine has a therapeutic index of only 1 : 4. If plasmoquine is given for 6 days after blood inoculation, the first dose being given 4 hours after inoculation and the remaining doses at 24-hour intervals, the appearance of parasites in the blood of treated birds is very much delayed compared with the control untreated birds, or completely inhibited. The birds in which parasites do not appear may either harbour latent infections, or be "sterilised," in which case they can be reinfected with malaria as readily as clean birds. When the drug is given during the acute stage of the infection it causes a rapid decrease in the number of parasites, and after 24 hours those that remain appear vacuolated and degenerating. When birds are treated during the acute stage they are never completely freed from parasites, that is they are never sterilised. Plasmoquine when given 2 hours before blood inoculation has no prophylactic action, but a similar dose given 6 hours after inoculation greatly delays or inhibits the appearance of parasites. Roehl concludes that the action of plasmoquine is directly on the parasites and not through the formation of antibodies.

Roehl's results have since been confirmed and extended by a number of workers. Hegner and Manwell (1927) found that plasmoquine is very effective against bird malaria but does not free treated birds from parasites. Hegner, Shaw and Manwell (1928) studied the antimalarial action of a number of quinoline and other compounds in relation to their toxicity to *Paramoecium* and absorption by red blood corpuscles. Manwell (1930) studied the action of plasmoquine on *Plasmodium praecox*, *P. cathemerium*, *P. elongatum*, and *P. rouxi* and found that the different species react differently both to plasmoquine and to quinine; that the gametocytes are not more resistant than the schizonts; and that sterilisation is regularly obtained with plasmoquine in *P. elongatum* infections against which quinine is relatively ineffective, but sterilisation is never obtained in *P. cathemerium* infections. He considers that plasmoquine is not as superior to quinine as Roehl claimed. Later Manwell (1932) found that combined treatment of bird malaria with plasmoquine and quinine is not more effective than either drug separately; and that infections of *P. rouxi* are as susceptible to plasmoquine as are those of *P. elongatum* and

are more susceptible to quinine than are infections of any of the other species. He also found that plasmoquine has a direct but slow "plasmodicidal" action *in vitro*. The direct action of plasmoquine *in vitro* was previously shown by Borchardt (1930). Wampler (1930), contrary to the results of previous workers, found that plasmoquine acts mainly on the schizonts.

Kikuth (1931) found that, in spite of morphological and immunological differences, the various species of bird malaria react similarly to plasmoquine and quinine.

Fourneau and co-workers (1930) found that when given subcutaneously plasmoquine has a therapeutic index of 1 : 60 and consider that its action is indirect and that it does not sterilise. Russell (1931) found that intramuscular injections of plasmoquine for 7 days prevent infection by blood inoculation on the third day of treatment; whereas quinine does not prevent infection in similar circumstances.

Recently Russell and Nono (1932) and Tate and Vincent (1933) have shown that in bird malaria plasmoquine does not act as a prophylactic against mosquito infection. The latter have shown also that when parallel series of birds are infected by blood inoculation and by the bites of infected mosquitoes, and are treated with plasmoquine during the normal incubation period, the drug is very effective in the blood-inoculated cases but has no significant action in the mosquito-infected cases.

Since the discovery of plasmoquine, work on the chemotherapy of bird malaria has revealed several new synthetic drugs of possible value against malaria in man. Sergent and co-workers (1931) have published a preliminary note on the action of Fourneau "710" (N-diethylaminopropyl-8-amino-6-methoxy-quinoline) on bird malaria. Collier and Warstadt (1931) described the action of two compounds "R 118" and "R 123," the former of which they claim has a therapeutic index of 1 : 250 and the latter possibly greater still. Recently Kikuth (1932) has described the action of a new compound called "Atebrin" which promises to be of value in the treatment of human malaria. In birds, like quinine, it acts on the asexual cycle of the parasite and not on the gametocytes.

In the present work thirteen new synthetic quinoline compounds have been found to be active against bird malaria and a description of their action, compared with that of quinine and plasmoquine, follows.

II. METHODS.

The malaria parasite used in this work was a strain of *Plasmodium relictum* (Grassi and Feletti 1891) obtained from the late Dr Roehl of Elberfeld, and it is the strain with which his work on the action of plasmoquine on bird malaria was done.

The birds used for the experiments were all hens of the common German roller canary (*Serinus canarius*). The malaria was transmitted by direct blood inoculation from bird to bird. Blood was obtained from the vein running down

the inside of the leg and was collected in citrate-saline solution (sodium chloride 0·8 per cent., sodium citrate 1 per cent.). The birds to be inoculated each received 0·3 c.c. of the diluted blood injected into the pectoral muscle. Birds which were to be used in testing the antimalarial properties of compounds were always inoculated with blood from acutely infected birds showing parasites readily on direct blood examination.

Usually blood examination of the inoculated birds was begun on the fifth day. The blood films were stained with Leishman's stain and each slide was examined for a standard time of 3 min., and those in which parasites were not seen in this time were considered negative.

In the case of birds which developed normal attacks of malaria examinations were made daily from the fifth day until the end of the acute attack. In the case of birds in which the appearance of parasites was delayed or inhibited, examinations were made daily until the tenth day and thereafter at least thrice weekly until parasites were found, or for a period of about 6 weeks after inoculation. Birds of which the blood was negative for from 4 to 6 weeks were regarded as probably "sterilised" and were tested by reinoculation from acutely infected birds. In many cases birds were also tested for "sterilisation" by inoculating two clean birds from them about the thirtieth day, but this test was abandoned as it was found to be unreliable—sometimes blood from a bird which later developed malaria did not produce infection in either of the inoculated birds.

The compounds tested were fed directly into the stomach of the birds by Roehl's catheter tube method (Roehl, 1926). Compounds were given in solutions in distilled water or in fine suspensions in distilled water if they were not sufficiently soluble. The compounds were administered by means of a 1 c.c. tuberculin syringe the needle of which was cut off short and fitted with about 10 cm. of fine catheter tubing. The tube was inserted about 5 cm. into the bird's oesophagus so that the contents of the syringe were injected directly into the stomach.

The only point in which our technique differed from Roehl's is that while he gave a standard dose of 1 c.c. of solution per 20 g. of body weight of bird, we found it more convenient to give a standard dose of 0·5 c.c. of solution per 20 g. The quantity of solution was increased or decreased in proportion as the weight of the bird was above or below 20 g. We found this method of administering drugs very satisfactory, as it is simple, rapid, reliable and does not cause the birds treated any discomfort. Throughout the following experiments, which involved the use of over 500 birds of which 190 were treated with drugs, we had not a single death during manipulation. The deaths which appear in the tables were due either to intercurrent infections or to the birds having been chloroformed because they showed severe toxic symptoms.

For testing compounds the routine method of treatment was the same as Roehl's. Doses of the compound were given into the stomach on six consecutive days, the first dose being given 4 hours after the bird was inoculated

with malaria, and the other doses at 24-hour intervals. When testing a new compound, if possible, the maximum tolerated dose was first tried, but sometimes such a dose was not tolerated repeatedly, and it had to be reduced on subsequent days. The routine method was to include one untreated control bird to every four birds undergoing treatment with drugs.

III. Results.

A. *Malaria in untreated control birds.*

As this work extended over several years and was done in conjunction with routine testing of numerous possible antimalarial compounds it is impossible to give each series of experiments separately. Consequently, to save space, details of the birds treated with effective compounds are collected together in Table II, although it is realised that only birds inoculated at the same time with the same infected blood are strictly comparable. Nevertheless, the action of the effective compounds is so strongly marked within their therapeutic indices that minor differences in individual birds may be neglected. Similarly it is more convenient to bring together all the control birds and consider the behaviour of the malaria in them before dealing with the effect of treatment with the various compounds. In control birds we include all the birds which during the course of the experiments were inoculated from acutely infected birds, received no treatment with drugs, and were examined from the fifth day, or sooner, until the end of the acute stage of the attack of malaria. In all there were 211 of these control birds, and from Table I it is seen that 48 per cent. showed parasites in the blood on the fifth day or sooner, and that 99 per cent. were positive before the tenth day. Two birds among the hundreds inoculated from acutely infected birds failed to develop malaria. Thus, as in Roehl's work in testing antimalarial compounds, any delay in the appearance of parasites in the blood until the tenth day or later may be regarded as significant. In control birds the number of parasites increased to a maximum about the ninth to twelfth days and then gradually decreased until the birds went into the chronic state, with less than one parasite per microscopical field, about the thirteenth to twentieth days. In our experiments the mortality rate from malaria has been very low and did not exceed 2 per cent. in the control birds (Table I). Amongst twenty-one control birds which were examined for long periods only one showed a definite relapse.

In birds with chronic infections the parasite level varied greatly and some birds showed parasites at 25 per cent. of examinations while others showed no parasites in forty-six examinations. Generally only one parasite was seen in the positive examinations, so that the parasite level in chronic cases was well below one parasite per examination. We have no evidence in our experiments that the blood of chronically infected birds ever ceases to be infective, although in birds inoculated from chronically infected donors parasites usually did not appear until the eighth to tenth day, and, in some cases, one of two birds

inoculated at the same time did not develop malaria although subsequent reinoculation showed it to be susceptible to infection. In all, seven out of thirty birds inoculated from chronic cases failed to develop malaria.

Reinoculation of birds in the chronic state with blood from birds with acute infections of the same strain of malaria never resulted in definite acute attacks, but sometimes a few parasites were seen in the blood on subsequent days. This supports the view that birds with chronic infections of *P. relictum* cannot be reinfected with the same species, a fact which provides a valuable test for "sterility" in birds treated with antimalarial compounds (Et. and Ed. Sergent, 1921). If a definite acute attack supervenes after reinoculation of the treated bird it is good evidence that it had been cleared of malaria parasites after the primary inoculation, that is, the drug had "sterilised" the bird.

Table I. *Results of inoculation of* 211 *control birds.*

Total number of birds inoculated	211
Number which developed malaria	209 = 99 %
Number which never showed parasites in blood	2 = 1 %
Number of birds which died during acute stage of malaria	4 = 2 %

Time of appearance of parasites in the 209 *positive control birds.*

Days after inoculation	No. of birds positive	Percentage of birds positive
5	100	48
6	151	72
7	189	90
8	201	96
9	207	99
10–13	209	100

B. *The effect of various drugs on avian malaria.*

(*a*) *Quinine hydrochloride.*

As Goodson, Henry and Macfie (1930) recently investigated the effects of quinine and other cinchona alkaloids on the same strain of bird malaria we did not make extensive experiments with quinine. As is shown in Table II (Nos. 1–10) ten birds were treated of which two were sterilised, one (No. 1) by six doses of 10 mg. and the other (No. 8) by six doses of 2·5 mg. In our experiments birds varied greatly in their tolerance of quinine and most would not tolerate six repeated doses of 5 mg. The minimum effective dose is about 2·5 mg., so that the therapeutic index is 1 : 4 for birds which tolerate it well.

(*b*) *Plasmoquine* (Table II, Nos. 11–51).

It was found that some birds tolerate doses of 0·7 mg. for 6 days (Nos. 11–12) while others failed to tolerate 0·64 mg. for more than 2 days (Nos. 13–15), so that the highest safe dose is 0·32 mg. on six consecutive days. The smallest dose tried, 0·02 mg., was still markedly effective (Nos. 35–45), giving a therapeutic index of about 1 : 30, as Roehl found. The most noticeable feature of our experiments as shown in Table II is that we obtained a large

proportion of sterilised birds amongst those treated. We consider that a bird may be regarded as "sterilised" if no parasites are found in the blood for at least 30 days after inoculation, and if the reinoculation from an acute infection results in a definite acute attack of malaria. When reinoculated birds showed only a few parasites or if parasites did not appear until late, about the twelfth day, it is doubtful whether the parasites arise from a latent infection due to the primary inoculation or are the result of a low-grade infection produced by the second inoculation. In such cases the birds were regarded as "possibly sterilised." In some cases subinoculation into two clean birds from each suspected case of sterilisation was done, but later it was abandoned as unreliable. At present we know of no means of definitely deciding whether a bird is quite free from malaria parasites or is harbouring a very low-grade latent infection.

It is also apparent from Table II that the drug is almost equally effective throughout the range of doses tried—0·64, 0·32, 0·16, 0·08, 0·04, 0·02 mg.—and this is especially the case with doses of 0·04 mg. and upwards. In doses of 0·08 mg. or more, only one bird (No. 16) was found positive by direct examination, although reinoculation gave inconclusive results in three other cases (Nos. 17, 21, 22), and in one (No. 28) it indicated that a latent infection was present. In all cases where malaria developed in treated birds there was great delay in the appearance of parasites in the blood, and even with the minimum dose of 0·02 mg. they never appeared sooner than the twentieth day after inoculation.

The total number of birds sterilised, omitting doubtful cases, was sixteen out of thirty-three birds treated.

To test if our standard dose of 0·5 c.c. compared with the 1 c.c. standard of Roehl affected the efficacy of the drug an experiment was made by giving doses of 0·04 mg. in 0·5 c.c. of solution to three birds (Nos. 29–31), and giving the same amount of drug in 1 c.c. of solution to three more birds (Nos. 32–34). Six more birds were treated similarly with doses of 0·02 mg., three (Nos. 35–37) receiving 0·5 c.c. of solution and three (Nos. 38–40) getting 1 c.c. of solution. Although with the 0·04 mg. doses the drug appears to have been slightly more effective in the birds getting 0·5 c.c. of solution, with the 0·02 mg. doses the difference is slightly in favour of the 1 c.c. doses. Therefore, it can be concluded that the actual amount of fluid given is not important and may be neglected in comparing our results with those of Roehl.

Another important point is the time of giving the drug in relation to the time of inoculation. It is seen that in bird No. 14 one dose of 0·64 mg. given 4 hours after inoculation was sufficient to sterilise it. This must be borne in mind when comparing Roehl's and our results with those of Fourneau and co-workers (1930) and Manwell (1930) who did not give the first dose until the day after inoculation.

To test the prophylactic action of plasmoquine three birds (Nos. 46–48) were given four doses of 0·32 mg. on consecutive days and were inoculated

24 hours after the last dose; while three more birds (Nos. 49–51) were inoculated at the same time and then given similar doses 4 hours after inoculation and on the three succeeding days. The doses given previous to inoculation had no effect on the appearance of the malaria, parasites being found on the third to fifth days; while the four doses given after inoculation, although they did not sterilise, delayed the appearance of parasites in the blood until the seventy-fifth and seventy-sixth days.

A few birds were treated with plasmoquine during the acute malarial attack, and, as described by Roehl, a rapid decrease in the number of parasites in the blood ensued, and after 24 hours those present frequently had a vacuolated and degenerated appearance.

(*c*) *New synthetic quinoline compounds.*

Among the new quinoline compounds tested thirteen, prepared by Prof. Robinson and his collaborators[1] (Barger and Robinson, 1929), proved to be effective against bird malaria. Details of the comparative tests of these compounds with plasmoquine and quinine are given in Table II, from which it is seen that R 25 and R 36 are the most effective of the new compounds, and in efficacy are nearly on the level of plasmoquine. These two compounds will be considered first.

R 25, 8-*γ-aminopropylamino*-6-*ethoxyquinoline dihydrochloride.* The maximum tolerated dose is about the same as that of plasmoquine, 0·64 mg., and the minimum effective dose is 0·04 mg., giving a therapeutic index of 1 : 16 (Nos. 52–80), which is half that of plasmoquine. The sterilising action is marked in the larger doses (Nos. 52–63) and nine out of twelve birds were sterilised; but with smaller doses, although the appearance of parasites in the blood was inhibited or greatly delayed, sterilisation was not obtained. Altogether out of twenty-five birds treated, omitting one which died during the experiments, nine were sterilised. As with plasmoquine, treatment with this drug previous to inoculation had no prophylactic action (Nos. 78–80).

R 36, 8-*γ-aminopropylamino*-6-*methoxyquinoline dihydrochloride.* The action of this compound closely resembles that of R 25 except that it is more effective in smaller doses. The maximum tolerated dose is about 0·64 mg. and the minimum effective dose about 0·04 mg. (Nos. 90–119) giving a therapeutic index of 1 : 16. With this compound sterilisation was obtained with doses of 0·08 and 0·04 mg. (Nos. 100–110), and out of a total of 23 birds treated, omitting deaths during experiments, fourteen were sterilised. When given during the acute stage its action resembles that of plasmoquine: it produces rapid diminution in the numbers of parasites in the blood, and those present after 24 hours appear vacuolated and degenerate. It is not prophylactic if

[1] These compounds are: R 25, R 26, R 34, R 35, R 36, R 38, R 39, R 44, R 45, R 48, R 49, R 51 and R 52. The preparation of the first seven compounds, R 25–R 39, is described by A. W. Baldwin in Barger and Robinson (1929). The chemistry of the remaining compounds, R 44–R 52, has not yet been published.

administered previous to inoculation (Nos. 117–119). Except that its therapeutic index is 1 : 16, compared with 1 : 30, this compound compares favourably with plasmoquine in antimalarial action in birds[1].

R 26, *the butylation product of the side chain of R* 25. This compound appears to be less effective than R 25, although two out of the five birds treated were sterilised (Nos. 81–85).

R 34, 8-*β-aminoethylamino*-6-*methoxyquinoline dihydrochloride*, has a maximum tolerated dose of 0·64 mg., and sterilised with a dose of 0·32 mg., but 0·08 mg. caused only a short delay in the appearance of parasites (Nos. 86–87).

R 35, *the butylation product of R* 34, has a maximum tolerated dose of 0·64 mg., caused a delay to 24 days with a dose of 0·32 mg., and 0·08 mg. had no effect (Nos. 88–89).

R 38, 8-(*ε-amino-n-amylamino*)-6-*methoxyquinoline dihydrochloride*, is effective in inhibiting the appearance of parasites in the maximum tolerated dose, but has no action in doses of 0·08 mg. and less (Nos. 120–122).

R 39, *the product of normal butylation of the side chain of R* 38, has a delaying action in the maximum tolerated dose, but is even less effective than R 38 (Nos. 123–124).

R 44, 8-*γ-aminopropylamino*-6-n-*butoxyquinoline dihydrochloride*, has a maximum tolerated dose of 0·32 mg., a minimum effective dose of 0·04 mg., and a therapeutic index of 1 : 8 (Nos. 125–137). Its sterilising action is very marked in the higher doses and all the seven birds treated with 0·32 or 0·16 mg. were sterilised (Nos. 125–131); while the minimum effective dose, 0·04 mg., sterilised one out of three birds. Altogether, nine out of thirteen birds treated were sterilised, so that it is nearly as active as R 36.

R 45, *the product of butylation of R* 44 is less effective than R 44, and has a therapeutic index of only 1 : 4. Four of the twelve birds treated (Nos. 138–150) were sterilised.

R 48, 8-*β'-aminoethoxyethylamino*-6-*methoxyquinoline dihydrochloride*, has a maximum tolerated dose of 0·64 mg., a minimum effective dose of 0·08 mg. and a therapeutic index of 1 : 8 (Nos. 151–156). The sterilising action is confined to the higher doses of 0·64 and 0·32 mg. (Nos. 151–153), and two of the six birds treated were sterilised.

R 49, *the product of butylation of R* 48, unlike the butylation products of the other compounds tested, appears to be more effective than R 48. Its maximum tolerated dose, minimum effective dose and therapeutic index are the same (Nos. 157–162), but its action is more marked with doses of 0·08 mg., and three out of the six birds treated were sterilised.

R 51, 8-*γ-butylaminopropylamino*-6-*methoxyquinoline hydrochloride*, has a maximum tolerated dose of 0·32 mg., a minimum effective dose of 0·04 mg., and a therapeutic index of 1 : 8 (Nos. 163–176). While all doses within the therapeutic index cause delay of varying times in the appearance of the

[1] The compound R 36 has been tested by James, Nicol and Shute (*Proc. Roy. Soc. Med.* May 5, 1932: Plate I, cases 23 and 26) on malignant tertian malaria of man, but it appears to be inactive.

parasites, the sterilising action is less marked than in the more effective R compounds. Only three out of fourteen birds treated were sterilised.

R 52, *8-γ-propylaminopropylamino-6-methoxyquinoline hydrochloride*, has the same maximum tolerated dose, minimum effective dose, and therapeutic index as R 51 (Nos. 177–190), but is less active and only one out of the fourteen birds treated was sterilised.

Table II.

The amount of drug given to each bird is shown in the third column, where "6 × 10" means that six doses each of 10 mg. per 20 g. of body weight of bird were administered on six consecutive days. "1 × 0·64 + 5 × 0·32" means that after one dose of 0·64 mg. the amount of drug was reduced to 0·32 on the five following days owing to toxic symptoms produced by the larger dose. Unless otherwise indicated the first dose was given 4 hours after inoculation and the remaining doses at 24-hour intervals.

In the fourth column the numbers refer to the number of days after inoculation on which parasites were first found in the peripheral blood. "Sterilised?" means that the bird was possibly sterilised.

In the fifth column the figures denote the day after inoculation on which the bird was reinoculated, unless the figure is followed by "mths," when it means the number of months between the primary and secondary inoculations; "= +" means that reinoculation resulted in a typical acute attack; and "+ ?" means that reinoculation gave doubtful results; "0" means that reinoculation failed to cause the appearance of parasites in the peripheral blood.

In the sixth column the figures indicate the number of days after inoculation on which subinoculations into clean birds were made; "= 0" means that neither of the inoculated clean birds developed malaria; "= + 0" means that one of the two clean birds developed malaria while the other did not.

Drug	Bird No.	Doses No. × mg. per 20 g.	Day of appearance of parasites. Effect of treatment with drug	Reinoculation	Subinoculation
Quinine HCl	1	6 × 10	Sterilised	42 = +	30 = 0
	2	6 × 5	25	—	—
	3	5 × 5	8	—	—
	4	6 × 5	14	—	—
	5	6 × 5	14	—	—
	6	5 × 5	Died 6th day	—	—
	7	6 × 5	20	—	—
	8	6 × 2·5	Sterilised	49 = +	—
	9	6 × 2·5	13	—	—
	10	6 × 2·5	24	—	—
Plasmoquine	11	6 × 0·7	Sterilised	7 mths = +	—
	12	6 × 0·7	Sterilised	7 mths = +	—
	13	2 × 0·64	29	—	—
	14	1 × 0·64	Sterilised	45 = +	30 = 0
	15	2 × 0·64	Died 2nd day	—	—
	16	6 × 0·32	23	—	—
	17	6 × 0·32	Sterilised?	45 = + ?	30 = 0
	18	6 × 0·32	Sterilised	45 = +	30 = 0
	19	2 × 0·32	Died 2nd day	—	—
	20	6 × 0·16	Sterilised	45 = +	30 = 0
	21	6 × 0·16	Sterilised?	45 = + ?	30 = 0
	22	6 × 0·16	Sterilised?	45 = + ?	30 = 0
	23	6 × 0·08	Sterilised	45 = +	30 = 0
	24	6 × 0·08	Sterilised	45 = +	30 = 0
	25	6 × 0·08	Sterilised	45 = +	30 = 0
	26	6 × 0·04	Sterilised	45 = +	30 = 0
	27	6 × 0·04	Sterilised	45 = +	30 = 0

Table II (*continued*).

Drug	Bird No.	Doses No. × mg. per 20 g.		Day of appearance of parasites. Effect of treatment with drug	Reinoculation	Subinoculation
Plasmoquine	28	6 × 0·04		Latent infection?	45 = 0	30 = 0
	29	6 × 0·04	In 0·5 c.c. of solution	Sterilised	43 = +	—
	30	6 × 0·04		Sterilised	43 = +	—
	31	6 × 0·04		Sterilised	43 = +	—
	32	6 × 0·04	In 1 c.c. of solution	21	—	—
	33	6 × 0·04		30	—	—
	34	6 × 0·04		22	—	—
	35	6 × 0·02	In 0·5 c.c. of solution	21	—	—
	36	6 × 0·02		25	—	—
	37	6 × 0·02		32	—	—
	38	6 × 0·02	In 1 c.c. of solution	Sterilised	43 = +	—
	39	6 × 0·02		20	—	—
	40	6 × 0·02		22	—	—
	41	6 × 0·02		Sterilised	45 = +	30 = 0
	42	6 × 0·02		Sterilised?	45 = + ?	30 = 0
	43	6 × 0·02		Sterilised?	45 = + ?	30 = 0
	44	6 × 0·02		39	—	—
	45	6 × 0·02		Sterilised	6 mths = +	—
	46	4 × 0·32	Before inoculation	5	—	—
	47	4 × 0·32		8	—	—
	48	4 × 0·32		3	—	—
	49	4 × 0·32	After inoculation	75	—	—
	50	4 × 0·32		76	—	—
	51	4 × 0·32		76	—	—
R 25	52	6 × 0·5		Sterilised	4 mths = +	—
	53	6 × 0·32		Sterilised	5 mths = +	—
	54	6 × 0·32		Sterilised	30 = +	30 = 0
	55	6 × 0·32		Sterilised	30 = +	30 = 0
	56	6 × 0·32		Sterilised	30 = +	30 = 0
	57	6 × 0·16		24	—	—
	58	6 × 0·16		Sterilised?	30 = + ?	30 = 0
	59	6 × 0·16		Sterilised	30 = +	30 = 0
	60	6 × 0·16		Sterilised	30 = +	30 = 0
	61	6 × 0·16		Sterilised	30 = +	30 = 0
	62	6 × 0·16		28	—	—
	63	6 × 0·13		Sterilised	5 mths = +	—
	64	6 × 0·08		29	—	—
	65	6 × 0·08		Latent infection	30 = 0?	30 = + 0
	66	3 × 0·08		Died 3rd day	—	—
	67	6 × 0·08		Sterilised?	30 = + ?	30 = 0
	68	6 × 0·08		24	—	—
	69	6 × 0·07		25	—	—
	70	6 × 0·04		19	—	—
	71	6 × 0·04		8	—	—
	72	6 × 0·04		15	—	—
	73	6 × 0·03		Sterilised	5 mths = +	—
	74	6 × 0·02		7	—	—
	75	6 × 0·02		6	—	—
	76	6 × 0·02		7	—	—
	77	6 × 0·02		5	—	—
	78	4 × 0·32	Before inoculation	4	—	—
	79	4 × 0·32		2	—	—
	80	4 × 0·32		6	—	—
R 26	81	6 × 0·7		30	—	—
	82	6 × 0·5		Sterilised?	4 mths = + ?	—
	83	6 × 0·33		Sterilised	5 mths = +	—
	84	6 × 0·13		Sterilised	5 mths = +	—
	85	6 × 0·02		12	—	—
R 34	86	6 × 0·32		Sterilised	30 = +	30 = 0
	87	6 × 0·08		17	—	—
R 35	88	6 × 0·32		24	—	—
	89	6 × 0·08		7	—	—

Table II (*continued*).

Drug	Bird No.	Doses No. × mg. per 20 g.	Day of appearance of parasites. Effect of treatment with drug	Reinoculation	Subinoculation
R 36	90	6 × 0·7	Sterilised?	—	—
	91	6 × 0·32	Sterilised	30 = +	30 = 0
	92	6 × 0·32	Sterilised	30 = +	30 = 0
	93	6 × 0·32	Sterilised	30 = +	30 = 0
	94	6 × 0·16	Sterilised	30 = +	30 = 0
	95	5 × 0·16	Died 5th day	—	—
	96	6 × 0·16	30	—	—
	97	6 × 0·16	Sterilised	30 = +	30 = 0
	98	6 × 0·16	Sterilised	30 = +	30 = 0
	99	6 × 0·16	Sterilised	30 = +	30 = 0
	100	6 × 0·08	26	—	—
	101	2 × 0·08	Died 2nd day	—	—
	102	6 × 0·08	Sterilised	30 = +	30 = 0
	103	6 × 0·08	Sterilised	30 = +	30 = 0
	104	6 × 0·08	Sterilised	30 = +	30 = 0
	105	6 × 0·08	Sterilised	30 = +	30 = 0
	106	6 × 0·07	Sterilised?	—	—
	107	6 × 0·07	Sterilised	6 mths = +	—
	108	6 × 0·04	Sterilised	30 = +	30 = 0
	109	6 × 0·04	15	—	—
	110	6 × 0·04	Died 6th day	—	—
	111	6 × 0·03	8	—	—
	112	6 × 0·03	13	—	—
	113	6 × 0·02	11	—	—
	114	6 × 0·02	9	—	—
	115	6 × 0·02	9	—	—
	116	6 × 0·02	10	—	—
	117	6 × 0·32 } Before inoculation	5	—	—
	118	6 × 0·32 } Before inoculation	5	—	—
	119	6 × 0·32 } Before inoculation	5	—	—
R 38	120	6 × 0·7	Sterilised?	—	—
	121	6 × 0·08	7	—	—
	122	6 × 0·02	9	—	—
R 39	123	6 × 0·7	20	—	—
	124	6 × 0·02	7	—	
R 44	125	6 × 0·32	Sterilised	59 = +	39 = 0
	126	6 × 0·32	Sterilised	42 = +	30 = 0
	127	6 × 0·32	Sterilised	43 = +	30 = 0
	128	6 × 0·32	Sterilised	43 = +	30 = 0
	129	6 × 0·16	Sterilised	42 = +	30 = 0
	130	6 × 0·16	Sterilised	43 = +	30 = 0
	131	6 × 0·16	Sterilised	43 = +	30 = 0
	132	6 × 0·08	21	—	—
	133	6 × 0·08	Sterilised	43 = +	30 = 0
	134	6 × 0·08	18	—	—
	135	6 × 0·04	6	—	—
	136	6 × 0·04	Sterilised	43 = +	—
	137	6 × 0·04	10	—	—
R 45	138	6 × 0·32	28	—	—
	139	6 × 0·32	Sterilised	42 = +	30 = 0
	140	6 × 0·32	Sterilised	43 = +	30 = 0
	141	6 × 0·32	Sterilised	43 = +	30 = 0
	142	6 × 0·16	Sterilised	42 = +	30 = 0
	143	6 × 0·16	Died 7th day	—	—
	144	6 × 0·16	25	—	—
	145	6 × 0·08	30	—	—
	146	6 × 0·08	14	—	—
	147	6 × 0·08	21	—	—
	148	6 × 0·04	8	—	—
	149	6 × 0·04	6	—	—
	150	6 × 0·04	5	—	—
R 48	151	6 × 0·64	Sterilised	42 = +	30 = 0

Table II (*continued*).

Drug	Bird No.	Doses No. × mg. per 20 g.	Day of appearance of parasites. Effect of treatment with drug	Reinoculation	Subinoculation
R 48	152	6 × 0·32	Sterilised?	45 = + ?	30 = 0
	153	6 × 0·32	Sterilised	45 = +	30 = 0
	154	6 × 0·16	14	—	—
	155	6 × 0·08	11	—	—
	156	6 × 0·08	11	—	—
R 49	157	6 × 0·64	Sterilised	42 = +	30 = 0
	158	6 × 0·32	Sterilised	45 = +	30 = 0
	159	6 × 0·32	Sterilised?	45 = + ?	30 = 0
	160	6 × 0·16	Sterilised	42 = +	30 = 0
	161	6 × 0·08	Sterilised?	45 = + ?	30 = 0
	162	6 × 0·08	38	—	30 = 0
R 51	163	1 × 0·64 + 5 × 0·32	Sterilised	91 = +	30 = 0
	164	6 × 0·16	Sterilised	58 = +	—
	165	6 × 0·16	33	—	—
	166	6 × 0·16	31	—	—
	167	6 × 0·08	19	—	—
	168	6 × 0·08	19	—	—
	169	6 × 0·08	Sterilised	58 = +	—
	170	6 × 0·04	14	—	—
	171	6 × 0·04	25	—	—
	172	6 × 0·04	13	—	—
	173	6 × 0·04	14	—	—
	174	6 × 0·02	12	—	—
	175	6 × 0·02	9	—	—
	176	6 × 0·02	6	—	—
R 52	177	1 × 0·64 + 5 × 0·32	39	—	—
	178	1 × 0·16	7	—	—
	179	6 × 0·16	Sterilised	58 = +	—
	180	6 × 0·16	28	—	—
	181	6 × 0·08	27	—	—
	182	6 × 0·08	23	—	—
	183	6 × 0·08	14	—	—
	184	6 × 0·04	14	—	—
	185	6 × 0·04	23	—	—
	186	6 × 0·04	15	—	—
	187	6 × 0·04	23	—	—
	188	6 × 0·02	6	—	—
	189	6 × 0·02	5	—	—
	190	6 × 0·02	6	—	—

In Table III the more effective of the R compounds and plasmoquine are compared as regards the sterilising action of the various doses; the ratio of

Table III. *Comparative sterilising action and therapeutic indices of plasmoquine and some R compounds.*

Drug	Dose in mg. per 20 g. body weight: 0·64–0·7 St./tr.	0·32 St./tr.	0·16 St./tr.	0·07–0·08 St./tr.	0·04 St./tr.	0·02 St./tr.	Therapeutic index	Total St./tr.
Plasmoquine	3/4	1/3	1/3	3/3	5/9	3/11	1 : 30	16/33
R 25	—	5/5	4/7	0/6	0/3	0/4	1 : 16	9/25
R 36	1/1	3/3	4/5	5/7	1/2	0/4	1 : 16	14/22
R 44	—	4/4	3/3	1/3	1/3	—	1 : 8	9/13
R 45	—	3/4	1/2	0/3	0/3	—	1 : 4	4/12
R 48	1/1	1/2	0/1	0/2	—	—	1 : 8	2/6
R 49	1/1	1/2	1/1	0/2	—	—	1 : 8	3/6
R 51	—	1/1	1/3	1/3	0/4	0/3	1 : 8	3/14
R 52	—	0/1	1/3	0/3	0/4	0/3	1 : 8	1/14

St./tr. = Sterilised/treated.

sterilised birds to the total number treated; and the therapeutic indices of the compounds.

C. *The formation of methaemoglobin* in vitro *through the action of plasmoquine and two other quinoline compounds.*

It is known that one of the symptoms of plasmoquine poisoning in man is the appearance of methaemoglobin in the blood (Orachowatz, 1928). This phenomenon has been investigated experimentally in animals and *in vitro* by Le Heux and de Lind van Wijngaarden (1929). Accordingly we studied the comparative action of plasmoquine and the compounds R 25 and R 36 in this respect. We failed to produce methaemoglobin *in vivo* in canaries or in mice; but *in vitro* it was readily formed by all three compounds. The formation of methaemoglobin was investigated *in vitro* in blood of man, pig, ox, sheep and canary, and in all these animals similar results were obtained. In most of the experiments the methaemoglobin was estimated spectroscopically (for the spectroscopic determinations we are indebted to Prof. D. Keilin), but in some experiments quantitative estimations were made by comparing the oxygen capacity of normal and treated blood in Barcroft's differential manometers.

The results of all the experiments showed that the three compounds readily form methaemoglobin *in vitro* in dilutions of 1 in 2000. The minimum

Experiment No. 10. *Methaemoglobin estimated spectroscopically.*

Blood = washed red blood corpuscles of ox, diluted to 25 per cent. with Ringer's solution.

Compound and concentration	Methaemoglobin			
	20 min.	40 min.	17 hours	23 hours
Control	0	0	0	0
Plasmoquine 1/10,000	0	0	+	+ +
R 25 1/10,000	0	0	+ +	+ + + +
R 36 1/10,000	0	+	+ + + + + +	+ + + + + + + + + +
Control	0	0	0	0
Plasmoquine 1/50,000	0	0	0	Trace
R 25 1/50,000	0	0	0	+
R 36 1/50,000	0	0	+	+ +

Experiment No. 21. *Quantitative estimation of methaemoglobin in Barcroft's differential manometers.*

Blood = washed red blood corpuscles of ox, diluted with equal volume of Ringer's solution.
Concentration of compound = 1 in 2000 of above fluid.
Control = equal quantity of distilled water added in place of compound.
Methaemoglobin calculated in percentage, taking control equal to 100 per cent. haemoglobin.

Compound	Time	Haemoglobin %	Methaemoglobin %
Control	3 hours	100	0
Plasmoquine	,,	71	29
R 25	,,	60	40
R 36	,,	49·7	50·3
Control	23 hours	100	0
Plasmoquine	,,	37·5	62·5
R 25	,,	23·7	76·3
R 36	,,	20·7	79·3

concentration of plasmoquine which forms methaemoglobin is about 1 in 50,000. The lower limit was not determined for the other compounds, but it is less than 1 in 50,000. R 36 is the most active of the three in causing the formation of methaemoglobin *in vitro*, and R 25 is intermediate between R 36 and plasmoquine. Details of two experiments are given in both of which ox blood was used.

IV. Discussion.

The results we obtained with the control untreated birds agree in general with those of previous workers, except that in our experiments the mortality from malaria has been extremely low, less than 2 per cent., while Et. and Ed. Sergent (1921) had 30 per cent. mortality during acute infections and Fourneau and co-workers (1930) had over 20 per cent. of fatal infections. These workers, however, used a different strain of *P. relictum*. We also found that, as previously observed by Sergent (1921) and Fourneau *et al.* (1930), a very small proportion, 1 per cent. or less, of birds may fail to develop malaria after inoculation with infected blood. Another point in which our results differ from some previous workers is that we rarely observed cases of genuine relapse, whereas Manwell (1929) in *P. inconstans* (= *P. relictum*) observed relapses in 30 per cent. of cases. Manwell, however, regarded the finding of even a single parasite on one day as being evidence of a relapse, while we consider that a bird to have a real relapse must show parasites over a number of consecutive days, in fact, must have a regular acute attack, although it may be much milder than the primary one. Our failure to produce reinfection with the same strain of malaria agrees with the observations of Et. and Ed. Sergent (1921), Lotze (1930), Kikuth and Tropp (1927), Kikuth (1931), and Gingrich (1932).

The results obtained with quinine agree with those of previous workers, Roehl (1926) and Goodson, Henry and Macfie (1930), that the maximum tolerated dose is about 5 mg. given orally, the therapeutic index 1 : 4, and the sterilising action slight. The results obtained with plasmoquine confirm Roehl's (1926), except that we found the safe maximum tolerated dose for repeated administration is 0·32 mg. or half that of Roehl. Plasmoquine in our experiments proved to be very much more effective than quinine, especially as regards its sterilising action, and in this respect our results support Roehl and differ from Manwell (1930). The comparatively large proportion of cases which were sterilised by treatment with plasmoquine in our experiments agrees with the results of Manwell (1930, 1932). Our results also support those of Roehl and Russell (1931) to the effect that plasmoquine given previous to blood inoculation has no prophylactic action in bird malaria.

With regard to the new compounds found to be effective against bird malaria, the most effective of them, R 25 and R 36, closely resemble plasmoquine in their action and efficacy and are closely related to it in chemical structure. The other R compounds are inferior to R 25 and R 36 in having smaller therapeutic indices (Table III), 1 : 4 or 1 : 8 compared with 1 : 16,

but the sterilising action of R 44 appears to be as great or greater than R 25, R 36 or plasmoquine. From the large number of closely related compounds which we tested and found to have no antimalarial action, it is evident that very slight changes in the chemical structure of such quinoline compounds may cause great changes in their therapeutic action.

V. Summary.

1. The results of previous workers on the action of plasmoquine on avian malaria, *Plasmodium relictum* (Grassi and Feletti, 1891), have been confirmed and extended. Sterilisation was obtained in a large proportion of birds (16 out of 33) treated during the normal incubation period.

2. Thirteen new synthetic quinoline compounds were found to have antimalarial action.

3. Two of these compounds, R 25 = 8-γ-aminopropylamino-6-ethoxyquinoline and R 36 = 8-γ-aminopropylamino-6-methoxyquinoline, have therapeutic indices of 1 : 16, are nearly equal to plasmoquine in potency and resemble it in action.

4. A third compound, R 44 = 8-γ-aminopropylamino-6-*n*-butoxyquinoline, has a therapeutic index of only 1 : 8, but its sterilising action is equal to or greater than plasmoquine, R 25 and R 36.

5. The remaining compounds have therapeutic indices of 1 : 8 or 1 : 4 and vary in their sterilising action.

6. Experiments are described which show the formation of methaemoglobin *in vitro* through the action of plasmoquine, R 25 and R 36. In this respect the order of activity of the compounds is R 36, R 25, plasmoquine.

We wish to express our thanks to Prof. D. Keilin, under whose direction this work was done, for his constant advice and help.

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MORPHOLOGICAL STUDY OF MALARIA PIGMENT IN OOCYSTS OF NATURALLY INFECTED *ANOPHELES*[1].

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(With 4 Figures in the Text.)

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I. Introduction.

Some thirty years ago Stephens and Christophers (1903) first drew attention to the significance of the pigment in the malaria oocyst and figured the different species of *Plasmodium*. The character of the pigment has been referred to from time to time in text-books, but few attempts appear to have been made to utilise it as a means of differentiating the species of *Plasmodium* occurring in naturally infected *Anopheles*. The writer in 1929, while carrying out an infectivity survey of the house-frequenting *Anopheles* of Kampala, Uganda, made camera lucida drawings of many pigmented oocysts, but discontinued the enquiry because of the difficulty experienced in interpreting the different species. Gordon, Hicks, Davey and Watson (1932) at Freetown, Sierra Leone, studied the pigment in oocysts occurring in *Anopheles* captured in dwellings, but they also abandoned the attempt to classify it on account of the same difficulty. After the publication of a small number (Gibbins, 1932), the drawings were filed for future reference. The recent publication of James, Nicol and Shute (1933) showing the characteristic pigment of *Plasmodium ovale* and giving comparative descriptions of all the species of human *Plasmodium* has, however, excited a new interest in the study and it is intended to deal more fully with the subject in the present paper.

[1] Dr W. H. Kauntze, M.B.E., Director, Medical and Sanitary Services, Uganda, has kindly given permission for the publication of this paper.

II. PUBLISHED DESCRIPTIONS OF MALARIA PIGMENT.

From the relevant literature on the subject, summarised in Table I, there would appear to be considerable variation in the character and arrangement of the pigment of the different species of *Plasmodium* affecting man.

There is general agreement that *P. vivax* has the lighter coloured and more delicate form of pigment which may be in the shape of yellow or brown rods and grains and (excepting James, Nicol and Shute (1933) who state that it is scattered throughout the parasite in no definite pattern) it is agreed that the pigment is generally arranged in a curved line. *P. malariae* would appear to have the darkest and largest pigment of all the four species of parasites, the pigment being invariably arranged in a clump. According to Christophers, Sinton and Covell (1928) it may be as light coloured as in *P. vivax*. The description of James, Nicol and Shute (1933) differs from the others. They state that the pigment resembles cocci whereas all the other authors remark upon its coarseness. Black pigment in the form of grains, described by the earlier authors as pepper-grains, occurs in *P. falciparum*. Colonel James and his co-workers found them few in number (about 10 or 12) and characteristically arranged as a streak across the oocyst or in a half-circle. The well-defined contour of the oocyst in this species is said to be an outstanding feature.

III. TYPES OF PIGMENT FOUND AT KAMPALA, UGANDA.

The drawings of oocysts from *Anopheles costalis*, *A. funestus*, and *A. theileri* var. *hancocki*, 204 in number, were made in the fresh state, after the stomach had been compressed by cautiously removing with filter paper some of the fluid beneath the coverslip; the same degree of compression was aimed at each time, but rupture of the stomach was avoided as far as possible. Oocysts showing each type of pigment represented in my collection are figured. They have been placed in groups which, as near as possible, correspond to the descriptions of the various authors for the different species. In the case of *P. malariae* the description of James, Nicol and Shute (1933) is not followed, as the pigment of this species is very striking and closely corresponds to the somewhat brief description of the earlier authors. There was no indication that a particular type of pigment was confined to any one species of *Anopheles*.

(*a*) *Group I.*

Fig. 1 depicts oocysts resembling those of *P. vivax*. The pigment in all cases was yellowish or light brown and consisted of delicate rods and grains. Careful focusing was usually necessary before the pigment was located. It was present in oocysts of all sizes and was noted on occasions confined within a small space when sporozoites had formed and the cyst was on the verge of rupture. In some instances it was arranged in a clump, in others it was seen as a single curved line, while sometimes several lines of fine rods were found crossing each other in a manner somewhat similar to the pigment of *P. ovale*.

Table I. *Relating to the character of the pigment in Human Malaria Parasites.*

Authority	*Plasmodium vivax*	*Plasmodium malariae*	*Plasmodium falciparum*	*Plasmodium ovale*
Stephens, J. W. W. and Christophers, S. R. (1903)	Yellowish or golden, in wisps	Coarse and in a clump	Clump resembling black pepper-grains	—
Wenyon, C. M. (1926)	Light brown, fine and not infrequently arranged in a curved line	Dark brown to black	Dark brown to black	—
Christophers, S. R., Sinton, J. A. and Covell, G. (1928)	Yellowish or golden in wisps, very delicate and in large cysts, often only to be seen by using 1/12 lens	Coarse and in a clump, black but sometimes light coloured as in *P. vivax* from which it may be difficult to differentiate	Clump resembling pepper-grains, considerably coarser than *P. vivax*	—
Knowles, R. (1928)	Fine wispy yellowish brown rodlets and grains, often arranged in a curved line instead of an irregular clump	Dense, black and in a coarse clump	Oocysts look like glass globes, sharp and with well-defined contour, highly refringent, and with considerable blackish haemozoin pigment in the form of pepper-grains	—
James, S. P., Nicol, W. D. and Shute, P. G. (1933)*	Grains of pigment numerous (more than 30 or 40) lighter brown than in any other species, thin rods and specks scattered throughout the parasite in no definite pattern	Grains of pigment numerous but larger, thicker and much darker than those of *P. vivax*. In shape more like cocci than rods or specks and tend to be bunched together in a clump round the contour of the cyst	Contour of the oocyst remarkably well defined. Grains of pigment few (about 10 or 12), large in size and black. Characteristically arranged as a streak across the oocyst or as a half-circle	Grains of pigment more like those of *P. malariae* than of *P. vivax*. Always arranged in one or other definite patterns of which the most common is two rows of dots crossing each other at right angles. This pattern is maintained up to 8th or 9th day of growth

* Up to 72 hours at 25° C.

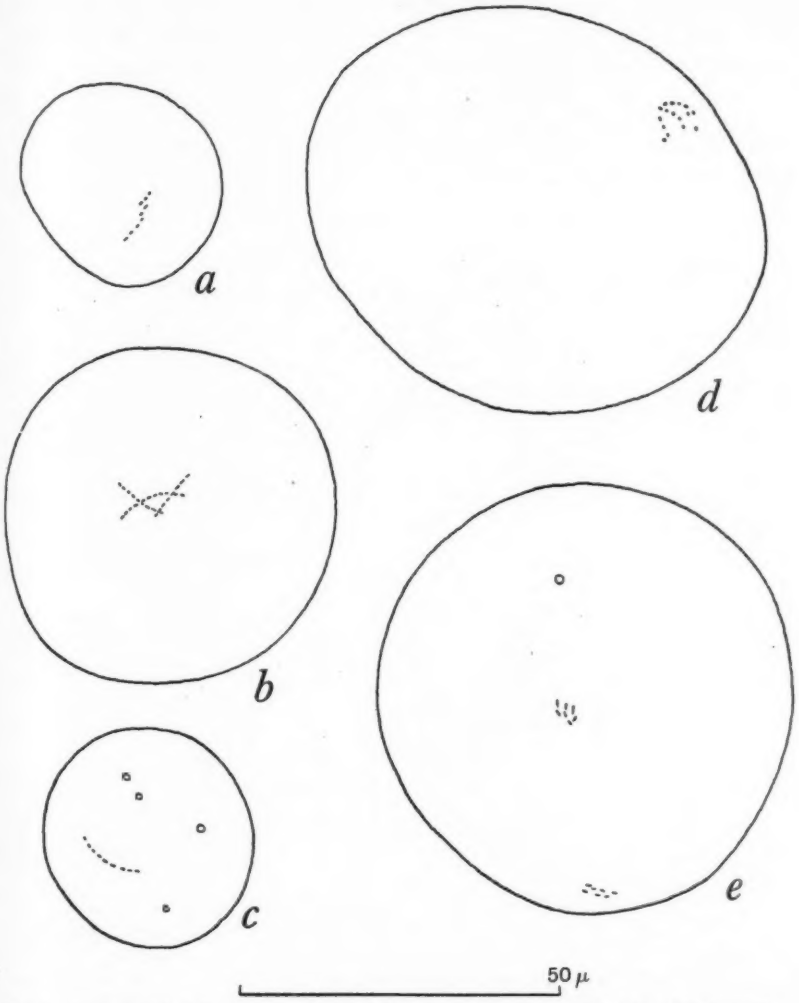

Fig. 1. Oocysts with delicate, yellowish to light brown pigment resembling that of *Plasmodium vivax*. *a*, one of nine oocysts from *A. costalis*. *b* and *d*, single oocysts from two different *A. funestus*. *c*, one oocyst from *A. costalis*. *e*, one of nine oocysts from *A. theileri* var. *hancocki*.

(*b*) *Group II.*

Fig. 2 comprises the dark brown to black pigmented oocysts which resemble those of *P. malariae* with the coarse and clearly visible irregular masses and rods, usually about 6–8 and occasionally up to 12 in number, commonly arranged in a curved or straight line, but sometimes in a clump, and on rare occasions scattered about the cyst. Examination under high magnification

revealed in many cases that the large masses of pigment were actually composed of a number of individual rods or granules closely adhering together. On one occasion this type of pigment was of a light brown colour.

(*c*) *Group III.*

The oocysts depicted in Fig. 3 all had a well-defined contour with pigment in the shape of black grains resembling that of *P. falciparum.* As belonging

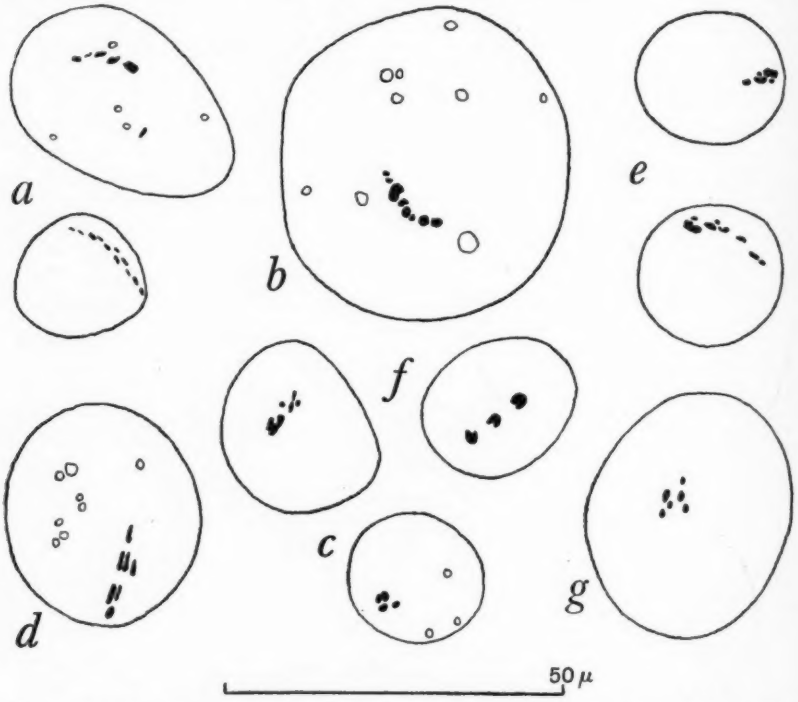

Fig. 2. Oocysts with coarse dark brown and black pigment resembling that of *Plasmodium malariae.* *a*, two oocysts from one *A. funestus.* *b*, one oocyst from *A. costalis.* *c*, two of nine oocysts from one *A. funestus.* *d*, one oocyst with three coarse masses of light brown pigment from *A. funestus.* *e*, one oocyst from *A. costalis.*

to this species is grouped pigment which is sometimes arranged in a curved line, but more frequently found in the form of a clump. In the younger cysts the latter type is generally lying free, later it is often collected within vacuoles which are almost a common feature in cysts of all sizes and more numerous in this, than the other groups. The number of grains of pigment was found to vary; in heavy infections they were few and sometimes absent altogether from some of the oocysts, while in others the pigment was represented by a few isolated grains.

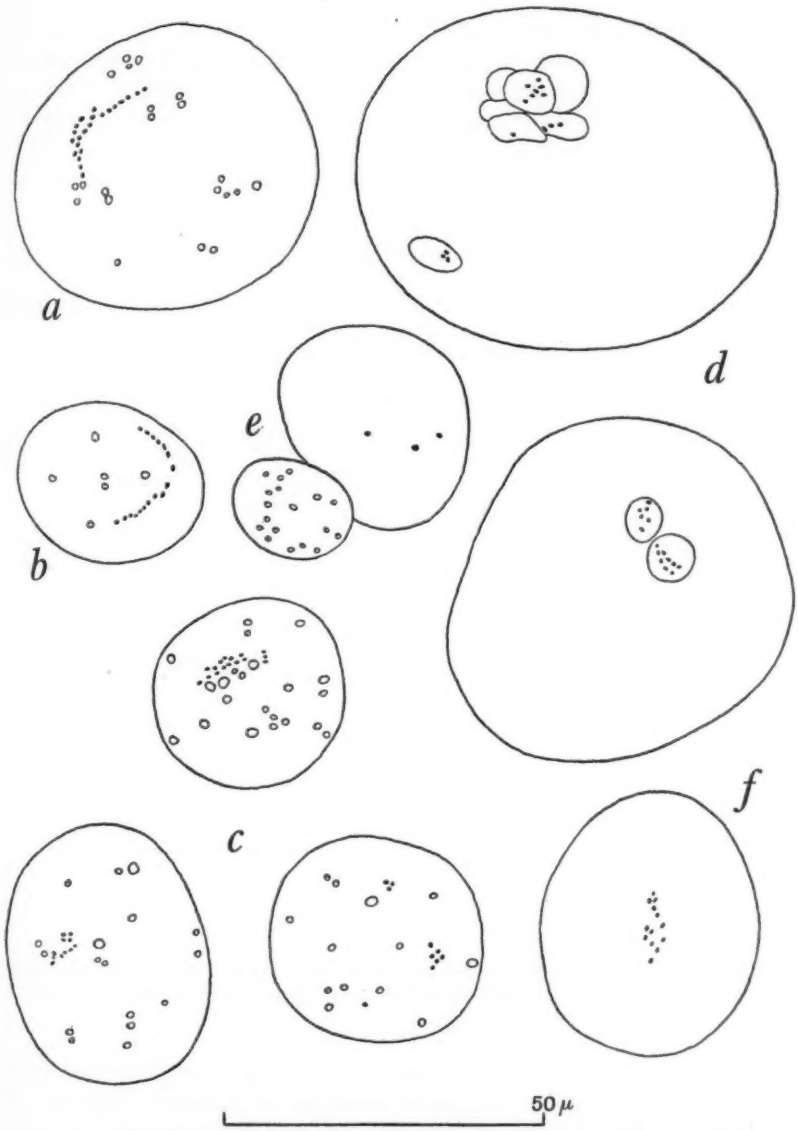

Fig. 3. Oocysts with black grains of pigment resembling that of *Plasmodium falciparum*. *a* and *b*, single oocysts from two different *A. funestus*. *c*, three of fourteen oocysts from one *A. costalis*. *d*, two of six oocysts from one *A. funestus* showing pigment within vacuoles. *e*, two of 152 oocysts from one *A. costalis* showing three grains of pigment in one cyst and none in the other which contains numerous vacuoles. *f*, one of two oocysts from *A. funestus*.

(*d*) *Group IV.*

Oocysts with pigment resembling *P. ovale*, illustrated in Fig. 4, were recorded by camera lucida drawings on four occasions, thrice in *Anopheles funestus* when one, two and four oocysts respectively were found, and once in *A. costalis* when a single specimen was present. The pigment was dark brown, shaped like a cocco-bacillus, and arranged in two or more curved lines which intersect each other about the centre of the oocyst. It was clearly visible and composed of 15–30 individual grains.

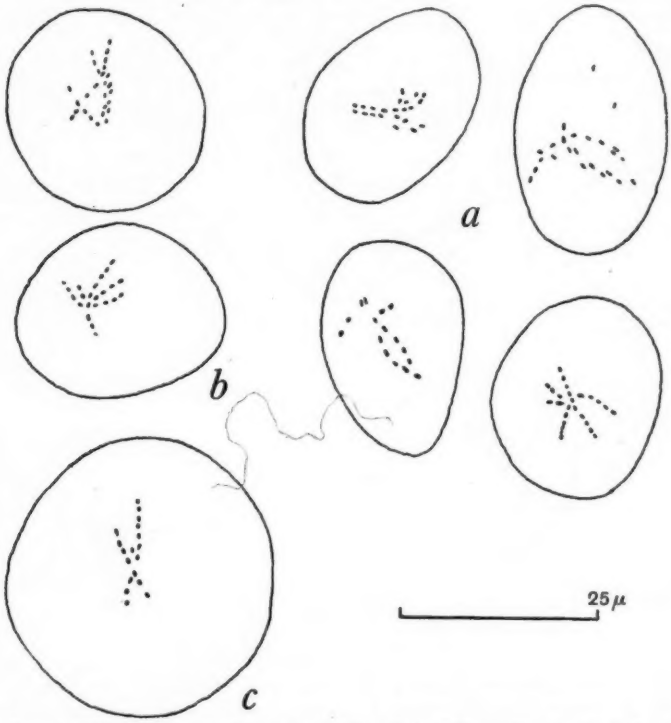

Fig. 4. Oocysts with dark brown pigment resembling that of *Plasmodium ovale*. *a*, four oocysts from the same *A. funestus*. *b*, two oocysts from one *A. funestus*. *c*, one oocyst from *A. costalis*.

IV. Summary.

The different types of malaria pigment occurring in oocysts of naturally infected *Anopheles* caught in human dwellings at Kampala, Uganda, are described and figured (selection from 204 camera lucida drawings).

The exact significance of each particular type of pigment is uncertain and in the present state of our knowledge it is not possible to do more than to place them tentatively into four groups and to indicate to which species they probably belong.

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RESEARCHES ON THE INTESTINAL PROTOZOA OF MONKEYS AND MAN

V. THE *ENDOLIMAX* OF MACAQUES.

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> Quod vero ad rerum vilitatem attinet, vel etiam turpitudinem,...eae res, non minus quam lautissimae et pretiosissimae in historiam naturalem recipiendae sunt....sol enim aeque palatia et cloacas ingreditur, neque tamen polluitur.—F. Bacon (1620), *Nov. Org.* lib. I, aph. cxx.

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Introductory note.

Some 14 years ago I summarized[1] all that had then been accurately ascertained about *Endolimax nana*. This little intestinal amoeba of Man had, at that date, been fairly well studied by many workers: its morphology and life-history were tolerably well known, though it had never been cultivated *in vitro*: and Man was then the only host it had been proved to inhabit.

When I began the present investigations in 1924 I discovered that Macaques, of more than one species, commonly harbour an *Endolimax* which is morphologically indistinguishable from *E. nana*. It had then just been recorded and roughly described by Brug (1923), who regarded it as a new species which he named *E. cynomolgi*. He found it at necropsy in a solitary specimen of "*Macacus cynomolgus*" [= *M. irus*] in Java—an old captive male monkey,

[1] Dobell (1919; p. 101 *sq.*). For earlier instalments of the present series of researches see Dobell (1928), Dobell with Bishop (1929), Dobell (1931).

used for many experiments during life. Both host and amoeba were obviously *species inquirendae*: and it must be noted that the same author had previously described and wrongly named an "*Endolimax kueneni*" from another monkey of the same species (Brug, 1921). Similar amoebae have since been seen and described—more or less—by others, whose records will be briefly considered later.

In the present paper I give a few observations on the species of *Endolimax* which I have found in various macaques and men, and some account of certain experiments which I have made with these organisms from time to time during the last 9 years. I have been particularly interested in the *Endolimax*-amoebae of Macaques because of their obvious resemblance to the species (*E. nana*) normally living in Man: and one of the main objects of my inquiries has been to find out whether or no the divers forms inhabiting these different hosts are specifically identical. My findings lead me to suppose that there is only one species of *Endolimax* which commonly lives in Man and monkeys of the genus *Macacus*, and my chief evidence for this conclusion is here presented.

Preliminary notes on these researches have already been recorded in earlier papers[1], and in the *Annual Reports of the Medical Research Council* for 1924–25, and 1925–26. My final results—up to the date of writing—are now published for the first time. In their entirety they include many observations and experiments made during the last 27 years (1906–1933). The researches are still in progress and unfinished, but my findings so far seem to me sufficiently definite to warrant publication. Work of this sort is endless, and therefore needs no apology for its incompleteness.

I. General observations and experiments.

(1) *Occurrence and distribution.*

It has already been recorded (Dobell and Laidlaw, 1926, 1926 *a*, and Dobell, 1928; p. 362) that I found an *Endolimax*, morphologically indistinguishable from *E. nana*, in *Macacus sinicus* [= *Macaca radiata*], *M. rhesus* [= *Macaca mulatta* = *Silenus rhesus*], and *M. nemestrinus*—the Indian Bonnet-monkey, the Bengal Macaque, and the Malayan Pig-tail. I have hitherto studied in detail only 10 specimens of these 3 species (4 *M. sinicus*, 5 *M. rhesus*, 1 *M. nemestrinus*), which are more particularly described in previous instalments (Dobell with Bishop, 1929; p. 448 *sq.*: Dobell, 1931; p. 7 *sq.* and Table I). From what has already been there recorded it will be evident that all my macaques were found to be naturally infected with *Endolimax*, with the sole exception of one male *M. rhesus* ("Jacko").

The amoebae in all these hosts were morphologically, culturally, and by every other available criterion, identical with one another, and indistinguishable from typical *E. nana* living in human beings. I spent much time in studying the amoebae in every monkey, but despite all my efforts I could never discover any character in which the *Endolimax* of any Macaque differed from

[1] Dobell (1928), Dobell and Laidlaw (1926, 1926 *a*), Dobell with Bishop (1929), Dobell (1931).

that of Man. As I have already described this species fully (Dobell, 1919) I need not repeat my description[1]. My previous account of *E. nana* is applicable, in every morphological detail, to the similar amoebae and cysts which I have since studied from *M. rhesus*, *M. sinicus*, and *M. nemestrinus*.

Whilst these researches have been in progress a few related records have been published by others. I may recall particularly, in this connexion, that Kessel (1928)—in China—has found an amoeba which he believes to be identical with *E. nana* in *Macacus rhesus*, *M. sancti-johannis*, *M. lasiotis*, and *M. irus* [= *cynomolgus*]. Other workers have observed similar forms in *M. rhesus* also, while Hegner and Chu (1930) have found what seems to be the same organism in *M. philippinensis*. All such findings will be discussed in more detail later.

All my monkeys which harboured *Endolimax* were studied continuously for long periods except No. 7 (Samson, *M. rhesus*), No. 9 (*M. rhesus*), and No. 10 (*M. nemestrinus*). These three animals were chiefly investigated *post mortem*, though I examined Samson's faeces and detected his infection microscopically during life. He was killed on 29. x. 24, and I found *Endolimax* in the contents of his colon and rectum—though I failed to find it in his caecum—at the necropsy[2]. Monkey No. 9 (as noted elsewhere[3]) had been dead for some time before I was able to examine her: but I found *Endolimax* in preparations made from the contents of her large intestine, and recovered it in cultures made from the same material (6. v. 25).

Monkey No. 8 (Delilah, *M. rhesus*) I had under observation for about 6 months. She was killed—by her owner, Dr W. Mair—on 27 January 1925, and I obtained her intestines for study immediately after death. The final results of a detailed examination—fresh and stained preparations, and cultures made from the contents of her caecum, colon, sigmoid, and rectum—showed the presence of *Endolimax* at every level in the large bowel.

Monkey No. 10 (*M. nemestrinus*) was killed by her owner (Sir Charles Ballance) on 3 August 1927. Her rectum was empty, but *Endolimax* was found in the contents of the rest of her large intestine, and recovered in cultures made from the caecum and colon[4].

My own monkeys which I sacrificed after prolonged investigation[5], and which I knew to be infected before death, all revealed the existence of *Endo-*

[1] I may note, in passing, that Stabler (1932) has recently reported that the nuclear structure of *E. nana* can be made to resemble that of an *Entamoeba* by a drastic method of fixation (Schaudinn's fluid adulterated with 20 per cent. of acetic acid). I rely upon results which I have obtained hitherto by more conservative methods.

[2] No cultures were made from this monkey, as I had not learnt how to cultivate *Endolimax* at that date.

[3] Dobell (1931), pp. 9–10.

[4] These initial cultures were made by Miss Ann Bishop, who kindly assisted me at the necropsy: but I made and studied the subsequent subcultures myself, and personally identified all the organisms in them.

[5] Namely, Bonar (No. 6), Polo (No. 1), Mungo (No. 3)—all *M. sinicus*—and Jacko (No. 2, *M. rhesus*), who was infected experimentally.

limax, microscopically or culturally, in every sample of the contents of their large intestines examined *post mortem*. In none of them could I discover any infection elsewhere: the small intestine appeared invariably to be uninfected.

From all my observations made hitherto, therefore, I conclude that *Endolimax* lives throughout the whole length of the large intestine in macaques, but nowhere else. By analogy, it may be inferred that the same distribution of *E. nana* obtains in human beings.

In all the monkeys examined, *Endolimax* was of course accompanied by other amoebae—including, in every case, *E. histolytica*. But hitherto no harmful effects attributable to any of these organisms have been recognizable during life, and no amoebic lesions have been discoverable *post mortem*. *Endolimax*, at all events, thus appears to be entirely non-pathogenic to its hosts[1].

I may add here that Hegner (1929) has lately recorded the occurrence of an *Endolimax* in the vagina of *M. rhesus*. The amoebae were discovered by Dr C. G. Hartman in vaginal washings from 8 monkeys, and are described as "indistinguishable morphologically from *Endolimax nana*." "The probability that these amoebae reached the vagina as a result of fecal contamination led to the study of fecal material from the eight infected monkeys": but Hegner succeeded in finding *Endolimax* in the faeces of only one individual, and therefore failed—as he says—"to clear up this situation." But apparently he made no cultures: and he certainly did not examine his monkeys sufficiently often to prove that seven of them had no intestinal infections[2].

I have now studied two female macaques, Susanna (*M. sinicus*) and Rosa (*M. rhesus*), thoroughly; and in the course of experiments with the former especially I have made daily examinations of the vaginal contents during long periods. I have found that it is very easy to contaminate the vagina with faeces in the process of obtaining material for study, and that in this way amoebae and flagellates may occasionally be introduced directly into it: while faecal material—including protozoa—is also often naturally introduced *in coitu*. But even though every precaution is taken to avoid such contamination, *Endolimax* (and *E. histolytica*) may occasionally be found inside the vagina—not merely near its orifice but even far in. As a result of daily microscopic and cultural examinations made repeatedly over considerable periods, I am satisfied that such amoebae have always wandered in of their own accord from the anus[3]. They never establish themselves in the vagina, and never encyst there—being stray organisms from the intestine and not natural vaginal inhabitants. They can be readily cultivated, however, together with concomitant bacteria of vaginal origin, and behave *in vitro* exactly like the intestinal forms in every way.

[1] This is further confirmed by the results of the two experimental infections—of Man and *M. rhesus*—recorded later in the present paper.

[2] According to his description he made only 24 examinations of the whole lot, and 3 individuals were examined only once apiece.

[3] The anatomical conformation of *M. rhesus* makes this particularly easy—apart from its dirty habits.

I therefore feel confident that if Hegner had studied his monkeys more carefully he would have found *Endolimax* living in the intestine of every animal. It seems highly improbable that this typically intestinal amoeba—which is so very common in the large bowel of *M. rhesus*—ever inhabits the vagina alone, where it appears to be incapable of existing for more than a brief period, where there is no evidence that it ever multiplies, and where it appears unable to encyst[1].

My own monkeys—few in number, but studied intensively for long periods of time—lead me to conclude that *Endolimax* occurs commonly in *M. rhesus* and *M. sinicus*, and also in other species of *Macacus*: and that it inhabits the large intestine, and lives normally in this situation alone.

(2) *Cultivation.*

I first succeeded in cultivating the *Endolimax* of *M. sinicus* and *M. rhesus* in the winter of 1924–25, using methods similar to those devised by Boeck and Drbohlav for *E. histolytica* in 1924. As all my monkeys were infected with the latter species, however, and as this grew more readily in all the media tried, I obtained at first mixed cultures only. After repeated attempts, and numerous failures, I obtained my first pure culture of *Endolimax* from *M. sinicus* in July–August 1925. This strain has already been briefly described (Dobell and Laidlaw, 1926 *a*); but as it figures largely in the present paper I shall here give further details, supplemented by the results of later observations and experiments.

The *Endolimax* of Macaques (*M. rhesus*, *M. sinicus*, and *M. nemestrinus*), like that of Man, is cultivable *in vitro* in all the media used successfully for growing *E. histolytica*, provided that the bacteria necessary for its nourishment are also present. *Endolimax* normally feeds upon bacteria exclusively—though it may ingest starch and other solid substances *in vitro*—and the species which it requires are still unknown. (I say this with confidence after 8 years' work and careful study of the literature.) A primary culture made with any of the usual media for intestinal protozoa, and from fresh faecal material containing cysts or amoebae, usually shows good initial growth together with many other organisms. Such mixed original cultures are therefore easy to obtain: the difficulty is to separate the *Endolimax* from accompanying amoebae of other species, flagellates, and *Blastocystis*, but along with those species of bacteria which are necessary for its own growth and complete development *in vitro* yet without those which are harmful.

All the monkeys from which I tried to isolate *Endolimax* were infected with *E. histolytica* and other protozoa, and therefore presented the usual difficulties. The first pure strain was obtained from my Macaque No. 4 (Susanna, *M. sinicus* ♀), who harboured—in addition to *E. histolytica* (2 strains, large and

[1] Even in cultures made from vaginal material neither *Endolimax* nor *E. histolytica* usually produces cysts. Such cultures often grow luxuriantly and strains *ex vagina* can be propagated *ad libitum*. Failure to form cysts is probably due to the circumstance that the requisite intestinal bacteria are lacking: for when these are added, encystation can be readily induced.

small) and *Endolimax*—*E. coli*, *Enteromonas*, and abundant *Blastocystis*. In the initial culture—made on 21 July 1925 by inoculating a fresh sample of Susanna's faeces into inspissated horse-serum medium covered with Ringer-eggwhite, with rice-starch[1]—all the foregoing organisms developed. I made some 25 subcultures during the next 10 days into various other media, and all of them but one series were failures. The series which succeeded was that propagated precariously for 5 generations in medium composed of inspissated horse-serum covered with sterile hay-infusion (instead of Ringer-eggwhite or dilute serum). The 6th serial subculture, made on 29 July 1925, produced apparently a pure growth of *Enteromonas*—accompanied by bacteria, of course, but free from *Blastocystis* and amoebae—and I inoculated it into Boeck-Drbohlav medium (solid egg, covered by dilute horse-serum) on August 1, hoping to obtain a pure culture of this flagellate, as the *Endolimax* had seemingly died out. To my surprise, I got instead a good mixed culture of *Enteromonas* and *Endolimax*, and after 4 days' incubation some of the amoebae encysted. I kept this culture at room-temperature for 24 hours, to kill off all the flagellates (which are very sensitive to cold), and then inoculated the surviving cysts into fresh egg-serum medium. On incubation they hatched excellently, and produced a "pure" culture (*i.e.* free from all other protozoa) of *Endolimax*. This culture, with several parallels made in similar ways, was the origin of Strain Σ—a pure strain of *Endolimax* from *M. sinicus*[2], with which most of the experiments about to be described were performed.

On further trial I found that the method which succeeded in this case is by no means infallible, so I do not advocate it as a general method for obtaining pure strains of *Endolimax* from an originally troublesome mixture of protozoa in the faeces of any macaque. One can never predict, with certainty, what will happen when any sample of faeces—containing known protozoa but an almost infinite number of unknown bacteria and other things—is incubated in any particular medium.

Strain Σ, when extracted pure, grew excellently in the modified Boeck-Drbohlav medium ("Ehs") which I generally use[3]. Very rich cultures were obtained, in which the amoebae were morphologically typical in every way—very few abnormal individuals having ever been seen in this strain. Moreover, after rapid multiplication for several days, many of the amoebae encysted; and abundant crops of characteristic quadrinucleate cysts—indistinguishable from those of *E. nana* found in human faeces—were easily obtained.

The course of development repeatedly observed in cultures of this strain was as follows: A tube of egg-serum medium inoculated with living amoebae and their concomitant bacteria showed a rich growth of amoebae after incuba-

[1] See Dobell and Laidlaw (1926 *a*). Since the appearance of this paper, no other workers—so far as I am aware—have added anything to our knowledge of the cultivation of *Endolimax*.

[2] The purity of this strain was proved by very careful study subsequently.

[3] Whole eggs thoroughly emulsified in Ringer's fluid, set as slopes by heating at 80° C. for about 1 hour, and then covered by horse-serum diluted with Ringer's solution (1: 8). See Dobell and Laidlaw (1926 *a*).

tion at 37°–38° C. for 24 hours. On continued incubation for another day abundant amoebae and a few cysts were usually found: by the third day the cysts had increased in number, and on the fourth they reached their maximum. Four-day cultures contained many thousands of typical ripe cysts, accompanied by a smaller number of active amoebae. On further incubation the results became somewhat irregular, both amoebae and cysts gradually dying at varying rates. Five-day cultures often contained good crops of normal cysts; but after a week most of them were dead, though sufficient amoebae still survived to be readily transplantable into fresh medium. Occasionally they lived for 10 days, but very seldom longer.

On continued incubation in the tube in which they were formed the cysts never appeared to hatch: they always degenerated and died. But if they were inoculated into fresh medium, they hatched readily and produced excellent cultures of normal amoebae which continued to develop as just described. I found that the cysts could live at ordinary room-temperatures for 2 or 3 weeks, but my experiments in this connexion will be described in the next section. All cultures, kept under constant conditions, behaved in exactly the same way. The bacteria present with this strain of amoebae appeared to be ideal for their multiplication, encystation, and excystation—without addition or subtraction: and I was thus able, for a time, to obtain all stages in the life-cycle of *Endolimax* at will *in vitro*.

Unfortunately this favourable state of affairs did not last. After a few weeks cysts began to appear irregularly in the cultures; and when the strain had been under cultivation for 2–3 months, I could obtain hardly any. The amoebae still grew splendidly and the cultures were as rich as ever, but they ceased to encyst. I tried every means I could think of to induce regular encystation once more, but failed completely: so on 31 January 1926, after continuous cultivation for 6 months, and when it was still growing luxuriantly and propagable in the amoeboid state *ad libitum*, I finally abandoned Strain Σ at its 51st cultural generation.

A similar loss of ability to form cysts I have observed repeatedly in numerous strains of *E. histolytica* and *E. coli* cultivated in various media. I attribute it to the dying out of some member (or members) of the concomitant bacterial flora, whose presence (or products) is necessary for encystation[1].

The addition of rice-starch to cultures of *Endolimax*—grown in the absence of this carbohydrate—has no influence in determining encystation. (This is in striking contrast with *Entamoeba histolytica* and *E. coli*, as already recorded[2].) Cultivated strains of *Endolimax* from Macaques and Man will sometimes ingest starch, but none which I have studied appears to need it for development: and, as everybody knows, *E. nana* amoebae in human stools almost

[1] Cf. Dobell and Laidlaw (1926 *a*), Dobell (1928), etc. Work on this very complex subject is still in progress, and evidence for my belief will be presented later.

[2] Dobell and Laidlaw (1926 *a*), Dobell (1928, 1931).

invariably contain no inclusions but bacteria[1]. I may add that neither Strain Σ nor any other strain of *Endolimax* which I have yet cultivated from any monkey or human being—and I have now tested many *in vitro* from various sources—would ingest human red blood-corpuscles. I have never seen a single individual of this genus, from any source, containing a blood-corpuscle—or any other living tissue-element—in its cytoplasmic vacuoles under natural or artificial conditions. I am therefore satisfied that *Endolimax* does not normally eat blood-corpuscles.

The *Endolimax* of Macaques (all strains studied), like that of Man, is very sensitive to changes of temperature. It will live and grow at about 36°–40° C., the optimum being 37°–38°. At 30° C. the amoebae can live for a considerable time, but below this temperature they soon round up and begin to degenerate[2]. Their resistance to temperatures above 40° I have not investigated.

The cysts of *Endolimax* have thinner walls than those of *E. histolytica* and *E. coli*, and they are therefore less resistant to antiseptics and other harmful chemicals. I have found, however, that they will usually survive treatment with *N*/20 hydrochloric acid for at least 1½ hours, and with a 0·1 per cent. solution of flavine for at least a day, at ordinary room-temperatures. These reagents kill most unencysted intestinal protozoa and *Blastocystis*, and I have frequently employed them to obtain "pure" cultures of *Endolimax*. As an example I may quote the following experiment from my notes:

Cysts of *Endolimax* (derived from Strain Σ) were found in fresh human faeces on 17. ix. 25, accompanied by a few amoebae and abundant *Blastocystis*. A sample of the stool was thoroughly emulsified in sterile distilled water, filtered through glass-wool, and the filtrate concentrated by repeated sedimentation at room-temperature. The final washed sediment—containing *Endolimax* cysts, dead amoebae, *Blastocystis*, and numerous faecal particles and bacteria—was then treated on 21. ix. 25 with *N*/20 HCl for 1 hour and 20 minutes. The suspension was then neutralized with 1 per cent. $NaHCO_3$ solution (using neutral red as an indicator), and again left to settle. On 24. ix. 25 —the cysts being then 7 days old, and apparently alive, in neutral solution— the sediment was inoculated into egg-serum medium (Ehs) and the culture put into the incubator (37° C.). After 2 days' incubation (26. ix. 25) active amoebae were found in this initial culture, but no other protozoa or *Blastocystis*. Two subcultures were made—one on 26. ix. 25, the other on 27. ix. 25. The first was successfully continued as a pure strain of *Endolimax* (Δ3): the second grew abundant *Blastocystis* as well as amoebae, and was therefore

[1] The same is true—though not previously noted—of the *Endolimax* amoebae commonly found in the faeces of macaques.

[2] The viability of the amoebae at room-temperature will be described in the next section: see p. 444. I can give no exact time for the survival of amoebae at temperatures between "room-temperature" (*ca.* 10°–20° C.) and that at which growth can take place (*ca.* 36° C.), because it varies so greatly with different strains and appears to depend chiefly upon the concomitant bacteria—most of which were undetermined, and therefore an unknown quantity in all my experiments.

discarded. This experiment proves that some ripe cysts of *Endolimax* can withstand treatment with $N/20$ HCl for $1\frac{1}{2}$ hours at room-temperature, but that most *Blastocystis* cannot. (Sufficient individuals remained alive to be recoverable by cultivation.) It also shows that *Endolimax* cysts are viable for at least a week at room-temperatures.

The whole of this experiment was made, of course, with every aseptic precaution—the sedimented cysts, with their accompanying faecal bacteria, being the only unsterilized ingredient. Consequently, the final cultures contained only *Endolimax* cysts (and a very few living *Blastocystis*) and those intestinal bacteria (or their spores) which survived treatment with the acid. These latter organisms are not always favourable for the growth of the amoebae: and I therefore found later that it is advantageous to inoculate the medium used for hatching such cysts with suitable bacteria previously. In later experiments of the same sort I used the mixture of bacteria accompanying Strain Σ (cultivated separately *en masse*, without any protozoa) for this purpose: and I then obtained much richer and better cultures of *Endolimax*.

Strain Σ is the only strain I have yet isolated which not only grew luxuriantly but also underwent encystation and excystation with regularity. During the first few weeks of its existence I obtained the entire life-cycle repeatedly *in vitro*—trophic amoebae in abundance, multiplying by binary fission; precystic amoebae, and cysts at all stages of development; excystation, and all the metacystic stages. I found that this organism (*Endolimax* from *M. sinicus*) develops in a similar way to *E. histolytica*[1], but owing to its extremely small size it is very difficult to study accurately. The metacystic stages, particularly, are excessively troublesome to unravel and interpret, and I am not yet satisfied with my observations. I hope, however, to complete them later, when I have been able to obtain another strain as good as Σ. Unfortunately, all others which I have since succeeded in isolating have behaved irregularly, most of them refusing to encyst; or, when they formed cysts, they hatched badly *in vitro*, or sometimes would not hatch at all[2]. From my work on *E. histolytica* and *E. coli* I am confident that all these many failures have been due to the presence of a concomitant bacterial flora unsuited to the complete development of the amoeba; but with further work I hope to strike the right micro-organisms again, and to complete my study of the life-cycle of *Endolimax* so that I can describe it in every cytological detail.

(3) *Viability of amoebae and cysts.*

For various practical purposes it is important to know how long amoebae and cysts can live when cooled to ordinary room-temperatures outside the body or the incubator. As a rule, the amoebae of *Endolimax* degenerate and die rapidly when they leave their host, though its ripe cysts may remain apparently

[1] See Dobell (1928).

[2] This applies both to the strains from macaques and to those obtained from human beings.

unchanged and normal for many days, or even several weeks, if they are not allowed to dry and are not exposed to any deleterious agent.

I have already pointed out (Dobell, 1927), in the case of *E. histolytica*, that trustworthy information on this subject can be obtained only by careful experimentation with cultures. The constitution of faeces is too uncertain and variable to afford any constant results. Cysts may live, for example, in one sample of faeces for weeks: in another for only days or even hours. I determined the viability of *E. histolytica*—both amoebae and cysts—by various methods; and with *Endolimax* I have made many similar experiments, which I shall now record.

As noted in the earlier paper (1927), it is by no means easy to obtain cysts or amoebae pure—that is, amoebae unaccompanied by any cysts whatsoever, or ripe cysts guaranteed free from admixture with amoebae. A good culture of amoebae, obtained after a certain period of incubation, may—and often does—contain cysts (not always easily discoverable): while a culture containing abundant cysts invariably contains amoebae as well. No conclusive experiments can be made with such mixtures. To make any experiments at all, to test relative viability, one must of course have a strain of amoebae which can be cultivated continuously under carefully controlled conditions, and which can be made to encyst and excyst at will *in vitro*. Hitherto I have been able to obtain only one such strain (Σ, already described), and I have performed nearly all my experiments with this.

Viability of cysts at room-temperatures. When a culture containing abundant cysts, accompanied by amoebae, is removed from the incubator, the amoebae soon become rounded up and motionless and in a few hours many of them are obviously degenerating. After 24 hours at room-temperature most of the amoebae appear to be undoubtedly dead, and after 2 days no living ones (apparently) can be found. Later they disintegrate, and ultimately disappear. By direct observation alone, therefore, it can be shown that *Endolimax* amoebae cannot live at room-temperature for more than a few days at most.

With cysts, however, it is very different. Ripe quadrinucleate specimens remain apparently normal and unchanged for at least a week in cultures kept at room-temperature: after 2, 3, or even 4 weeks some of them—in rapidly diminishing numbers—still appear to be alive. Their viability can be tested by inoculating them into fresh culture-tubes and seeing whether or no they can hatch and produce normal cultures of amoebae again under suitable conditions.

I made many experiments with culture-cysts of *Endolimax* (Strain Σ) along such lines. The most extensive series was made in August and September (1925), and it is summarized as an illustrative example in Table I. It should be remembered that the initial material, in each experiment, was a culture containing abundant ripe cysts accompanied originally by amoebae which died out (in each culture) at a time not precisely determined by these observations. Living, or possibly living, amoebae were present in the inoculum during the first 2 days, but thereafter they were all certainly dead. When primary cultures

inoculated with cysts showed no growth of amoebae after incubation for 48 hours, they were subinoculated into fresh tubes and the results of these subcultures were taken as final. (In the Table, the sign + denotes a normal growth of amoebae: and the sign − means that no amoebae were discoverable after prolonged search. The entry 0 indicates that no subculture was made.)

Table I. *Tests of viability of* Endolimax *culture-cysts* (*Strain* Σ) *at room-temperatures.*

Time for which cysts were kept at R.T.	Result of initial culture	Result of subculture	Remarks
24 hours	+	0	Cultures grown from amoebae as well as cysts?
26 „	+	0	
2 days	+	0	
3 „	+	0	
5 „	+	0	2 cultures made with same result
7 „	+	0	Ditto
9 „	+	0	Ditto
10 „	+	0	
11 „	+	0	
12 „	+	0	
13 „	+	0	
14 „	+	0	3 cultures made, 2 + 1 −
15 „	+	0	
16 „	−	−	2 subcultures made
17 „	−	+	
18 „	−	−	
19 „	−	−	
20 „	−	−	

If we discount the results obtained during the first 2 days, these experiments show that cysts of *Endolimax* live for a fortnight at least—under the conditions defined—at room-temperatures[1]. A few may survive for as long as 17 days, but none lived longer.

This conclusion is, however, true for this series of experiments only. At the time when I made these cultures I did not know (for I discovered it later, when experimenting with cysts of *E. histolytica*) that a constant low temperature[2] greatly favours the survival of cysts of all entozoic amoebae. At later dates I therefore repeated these tests with other strains of *Endolimax*: but all these trials were more or less unsatisfactory, because I have never succeeded in obtaining, in pure culture, any strain except Σ upon which I could rely. The cysts of all the others hatched irregularly, and I could not always cause their encystation *in vitro* at will. My later work has shown, however, that the cysts of *Endolimax*—derived from both Man and Macaques—can live at a constant temperature of about 10° C. for at least 3 weeks: and I believe that, under more favourable conditions, they can survive much longer. But I cannot prove this conclusively, at present, so give only my actual findings.

Viability of amoebae at room-temperatures. Experiments such as those

[1] In the experiments here described, "room-temperature" (*i.e.* the temperature in my laboratory) was very variable. It ranged, from day to day, from about 15° C. to something over 24° C.

[2] *Ca.* 5–10° C.

recorded in Table I throw no light upon the duration of survival of the amoeboid forms of *Endolimax* but only of its cysts. To test the viability of the amoebae at room-temperatures I used a method already described (Dobell, 1927) in the case of *E. histolytica*, as I have been unable to devise any other which will give cultures of amoebae only—certainly free from all living cysts—for such purposes. The method consists in causing encystation in a culture (in the present case, by incubating it at 37° C. for 4 days): then placing it at room-temperature for a time sufficient to kill off with certainty all amoebae accompanying the cysts (4 days or more): then hatching these cysts by inoculation into suitable medium and incubating them at 37° C. for 24 hours: and finally testing such cultures containing amoebae only (hatched from cysts, and before they have been able to encyst again) by subinoculation and cultivation in favourable media after keeping them at room-temperatures for various periods of time. Such experiments—with all due precautions—even with a trustworthy strain are extremely laborious to make. I have so far been able to perform only one thoroughly controlled series, and record its results in Table II. These experiments were all made with cultures of *Endolimax* (Strain Σ) in August 1925.

Table II. *Viability of* Endolimax *culture-amoebae* (*Strain* Σ) *at room-temperatures.*

Time for which amoebae were kept at R.T.	Result of initial culture	Result of subculture
8 hours	+	0
21 ,,	−	+
24 ,,	+	0
27½ ,,	−	−
44 ,,	−	−
52 ,,	−	−
3 days	−	−
4 ,,	−	−
5 ,,	−	−

The foregoing experiments show that some *Endolimax* amoebae can survive for as long as 24 hours when kept at room-temperature, but not for 27½ hours or longer: while most of them die within 21 hours. In subsequent work I have confirmed these observations repeatedly. Most of the amoebae die in an ordinary culture kept at room-temperature for a day, and one can be confident that all will be dead in 2 days. At lower temperatures they die more rapidly; and I have never found living amoebae in any culture kept in the ice-chest (*ca.* 10° C.) at the end of 24 hours.

A knowledge of the differential resistance to cooling of various species of intestinal protozoa is of great practical use, for it often enables one to separate species with ease and so obtain pure cultures[1]. For example, from a culture

[1] This has been pointed out already by Dobell and Laidlaw (1926 *a*), but I emphasize it here because I find many workers still believe that to obtain a *pure* culture of any desired intestinal protozoon from an animal harbouring several species—all growing in the same sort of media—is chiefly a matter of luck. Luck certainly plays a part, but skill and experience are of much more importance.

containing amoebae of *Endolimax* and *E. histolytica*, the latter can be obtained pure by cooling the mixture for a suitable time (about 24–30 hours at room-temperatures), and then inoculating the surviving amoebae into fresh medium and incubating at 37° C. A culture of *E. histolytica* mixed with *E. coli* can easily be purified in a similar way—the latter species being even more sensitive to cold than *Endolimax*. Similarly, if one needs cysts of any amoeba for experiment—guaranteed free from admixture with active forms—one can readily obtain them by keeping a culture containing cysts and amoebae in the ice-chest for a few days.

(4) *Susceptibility to emetine.*

Most of my observations under this head have already been published; but as the records are scattered, and incorporated in papers dealing with other subjects, it will be convenient to collect them here.

In studying the specific action of emetine upon *Entamoeba histolytica*, many observations have been made incidentally on the effects of this alkaloid upon *Endolimax*—both of Man and of Macaques. Briefly, the results have shown clearly that emetine has no specific action upon *Endolimax* either *in vivo* or *in vitro*.

As an instance of the action *in vivo* of emetine on human infections with *Endolimax nana*, I may quote some findings recorded 15 years ago[1]. A series of 155 patients infected with *E. histolytica* was treated—under very carefully controlled conditions—with emetine in sufficient dosage to cure the great majority. Of these patients, 52 were also infected with *E. nana*, but in no case had the treatment any demonstrable effect upon this organism. All the patients remained infected after administration of the alkaloid.

Comparable observations have been made upon macaques infected with *Endolimax*[2]. I have now given 8 courses of treatment with emetine, in sufficient quantity to eradicate their *E. histolytica* infections completely, to 4 of my monkeys (Polo, Mungo, Susanna, and Rosa—3 *M. sinicus* and 1 *M. rhesus*), but in no case has the treatment affected their concomitant infections with *Endolimax*. Two of these macaques (Susanna and Rosa) have each been treated thrice, with the same result. Another monkey also (Jacko, *M. rhesus*), who had been experimentally infected with *Endolimax* previously, received a large quantity of emetine in an unsuccessful attempt to cure his natural *E. histolytica* infection: but here likewise the result was negative.

All the evidence which I have yet obtained indicates that the *Endolimax* of Man and that of Macaques both react in exactly the same way towards emetine. It seems impossible to eradicate an infection with these amoebae by any dosage which either host can tolerate.

Experiments made with emetine on amoebae *in vitro* have explained these

[1] See Dobell, Gettings, Jepps, and Stephens (1918).
[2] See Dobell with Bishop (1929), and Dobell (1931).

results. It has been found[1] that *E. histolytica* is extremely sensitive to the alkaloid, but that *Endolimax* is not: and it is thus possible to imitate in the test-tube the effects of emetine-treatment on a man or macaque infected with both species. When a mixed culture of *E. histolytica* and *Endolimax* is treated with emetine in suitable dilution, the former is killed while the latter survives; so that one can easily isolate a pure culture of *Endolimax* in this way from such a mixture[2].

All the observations and experiments which have been made and recorded hitherto reveal no essential difference between the *Endolimax* of Man and that of Macaques in their reactions towards emetine *in vitro* or *in vivo*: on the contrary, they serve to emphasize their resemblance, and thus furnish additional evidence for specific identity.

So far as I am aware, no alkaloid or other chemical compound having a specific action upon any *Endolimax* has yet been discovered. I must note, however, that Kessel (1928) claims to have eradicated spontaneous infections with "*E. nana*" from 2 macaques (*M. rhesus*) by treatment with "yatren" of unspecified dosage for an unrecorded period: while Anderson and Koch (1931) make similar claims for the efficacy of a somewhat similar substance ("vioform")[3]. Another worker (Faust, 1930), who apparently believes that "dihydranol" is a panacea for intestinal protozoal infections, alleges that he has removed *E. nana* from 2 human beings by treatment with this drug. After reading the reports of all these American workers, I can only conclude that there is still no convincing evidence to support their beliefs. Making every allowance for obvious omissions and inaccuracies in these still imperfectly published records, I nevertheless find nothing to indicate that anybody has yet discovered any chemical substance which has a specific lethal action upon any known species of *Endolimax* whatsoever—either *in vivo* or *in vitro*.

II. Particular animal experiments.

(1) *Experimental transmission from* M. sinicus *to Man.*

When I began these researches, in 1924, no attempt had ever been made to infect any human being experimentally with the *Endolimax* of macaques, or any monkey with *Endolimax nana* from Man. But if these amoebae are specifically identical, they should obviously be transmissible from one host to the other: and in 1928 Kessel stated that he had successfully infected macaques

[1] Dobell and Laidlaw (1926). See also Laidlaw, Dobell, and Bishop (1928).

[2] Dobell and Laidlaw (1926), pp. 216–17. The toxicity of emetine for *Endolimax* has not yet been as exactly determined as it has been for *E. histolytica* (Laidlaw, Dobell, and Bishop, 1928) and for *E. coli* (Bishop, 1929): but it is certain that this amoeba can usually tolerate at least four times the amount of the alkaloid which is fatal, in culture, to *E. histolytica*.

[3] From their paper it can be inferred that they treated altogether 6 infected macaques (2 *M. rhesus* and 4 *M. cynomolgus*) with vioform "in divided doses over 3 to 6 weeks," giving "a total dosage of 900 to 1200 mgm. per kilo." Of these monkeys, 3 (1 *M. rhesus* and 2 *M. cynomolgus*) are registered as negative for "*E. nana*" 3 months after treatment: the 3 others were still infected.

by feeding them on *E. nana* (of human provenance). The reverse experiment (of which he was apparently ignorant)—infection of Man with the *Endolimax* of *M. sinicus*—I had attempted previously in 1925, and the result was recorded in the *Annual Report of the Medical Research Council* for 1925–26. I now give it in greater detail.

The chief obstacles in the way of attempting to infect human beings with the protozoa of monkeys—or other animals—are three: (1) the difficulty of finding a willing subject for experimentation; (2) the difficulty in proving that any such subject is not naturally infected with any organism indistinguishably similar to the one used for the experiment; and (3) the difficulty of obtaining the desired stages of the requisite protozoon in pure culture. I have already described how I surmounted the last obstacle by obtaining from *M. sinicus* a pure strain of *Endolimax* (Σ) which I could—for a time—cause to encyst *in vitro* at will. The two other difficulties were overcome by using myself as the experimental host.

I have now made various attempts to infect myself with divers intestinal protozoa. These experiments will all be described in due course; but as this is the first to be recorded fully, I must now enter into my protozoological history in some detail and give evidence to show that I was uninfected with *Endolimax nana* at the time when it was performed.

Personal data. I have been keenly interested in entozoic amoebae ever since I first commenced my researches on intestinal protozoa in the year 1906. In the winter of 1906–7, when I was 20 years of age, I began my studies of *Entamoeba ranarum* and other protozoa of frogs: and in order to find out whether I myself harboured any similar intestinal protozoa, I then examined my stools microscopically for the first time. The results were negative: I could discover no protozoa of any kind after careful search on at least half-a-dozen occasions. Unfortunately I can give no more precise information regarding these early examinations, as I kept no proper record—feeling, at the time, that these were matters which "as Pliny saith, require apology for even mentioning them[1]." I had not then learnt from Leeuwenhoek, who was the first man to describe protozoa from his own faeces[2], that modesty is misplaced in the registration of scientific fact—"*cum hoc genus fastidii sit plane puerile et effoeminatum*[3]." But with such grand justifications I can now describe my own case unblushingly.

I examined my own stools again microscopically in 1907, 1908, and 1909, but still failed to find any protozoa in them. But in 1910 (I have kept no record of the exact date) I discovered that I was infected with *Giardia*, after various visits to the continent of Europe (Germany, Italy, and Bohemia) and a short sojourn in Ceylon. At the end of 1915, when I began to study the in-

[1] Bacon, *Nov. Org.* I, Aph. 120. Another fragment of this remarkable passage is quoted in the motto at the head of the present instalment.

[2] Cf. Dobell (1932), p. 222 *sqq.*

[3] See note 1 above.

testinal protozoa of Man in earnest, I subjected myself to exhaustive examination: and between 20 December 1915 and 14 August 1925 I examined my stools microscopically, with great care, more than 200 times. (The examinations which I have recorded are 174 in number, but I made many more which were not entered in my notes because they were completely negative.) My object in making these examinations was primarily, at that time, to ascertain whether or no I might accidentally acquire infection with any intestinal protozoa in the course of my work—which involved the handling of many thousands of samples of human stools—though I took every precaution to avoid such a contingency: but I also examined myself in order to study the course of my natural infection with *Giardia*, and to find out how long it would persist.

In August 1925, although I was fully satisfied by previous microscopic examinations alone that I harboured no intestinal amoebae of any sort, I made a few cultures—by methods which I had then fully tested—from my stools. The results were again all entirely negative. Consequently, I considered that I was, for experimental purposes, a "clean animal." During nearly 20 years I had made more than 200 unsuccessful attempts to discover a possible infection with amoebae; and I think the evidence for my non-infection at the date of the experiment is conclusive. At all events, no other worker—to the best of my knowledge—has ever adduced stronger evidence for the non-infection of any animal used in any similar experiment with any intestinal protozoon. Moreover, I had never suffered from dysentery, persistent diarrhoea, or any other intestinal disorder, and I was—as I still am—a physiologically normal and structurally intact human being as far as my alimentary canal is concerned. I was therefore an almost ideal subject for infection.

The material used for the experiment was a culture of *Endolimax* (Strain Σ, derived from *M. sinicus*) in which cysts alone were present. It was the 11th serial subculture of this strain in "Ehs" medium made on 14 August 1925 and removed from the incubator on August 18, when it contained abundant ripe cysts and many amoebae. I kept this culture at room-temperature for a week, in order to kill all amoebae with certainty[1]. On August 25, when it contained numerous apparently normal and ripe living cysts, and only a few dead amoebae, I inoculated a small amount of it into a fresh culture-tube and put it in the incubator (to test the vitality of the cysts): and I then swallowed the rest of the cysts myself.

The experiment was made as follows. All the cysts contained in the sediment in culture Σ (11) Ehs (14. viii. 25) 18. viii. 25 were mixed—with their concomitant bacteria—into a semiliquid mass with sterile rice-starch: and this material was then put into two small gelatin capsules which I swallowed with a draught of water on an empty stomach. I was careful to prevent the mass drying, as I knew this would kill the cysts, but I made no attempt to determine their number precisely. Several thousands, at least, were ingested with the capsules. That many of these cysts were alive and capable of further develop-

[1] Cf. p. 447 *supra*.

ment was proved by the control culture inoculated with a small sample, which showed an abundant pure growth of typical *Endolimax* amoebae 2 days later (27. viii. 25).

After swallowing the cysts on August 25 I examined my stools every day, both microscopically and culturally. They remained microscopically negative for *Endolimax* until 5 September 1925, when I discovered a few active amoebae and cysts in them for the first time. But the cultures made on the previous day (4 September 1925) contained *Endolimax* amoebae after incubation for 24 hours, so that infection was first demonstrable 10 days after inoculation.

I continued the daily microscopic and cultural examinations for another 13 days (till 18. ix. 25), and they were all—except one negative microscopic (but culturally positive) examination on 15 September 1925—positive for *Endolimax*. Since then I have examined my stools for various periods, at various intervals of time, and most of the examinations have proved positive—demonstrating that the infection has persisted to the present day. The precise figures to date (June, 1933) are as follows: 210 examinations made, both microscopically and culturally, since 5 September 1925; whereof 158 direct microscopic examinations were positive and 52 negative. Cultures have been almost invariably positive[1].

There can thus be no reasonable doubt that the experiment was a success, and that the infection was firmly established. For at least 19 years before the experiment was performed I was uninfected with *Endolimax* (as proved by more than 200 consecutive negative examinations): while 10 days after ingestion of cysts the infection was demonstrable, and it has remained so ever since for some 8 years (over 200 positive examinations). It still shows no sign of dying out. I summarize the whole experiment in Table III.

Table III. *Infection of Man by means of culture-cysts of* Endolimax (*Strain* Σ) *from* M. sinicus. *Cysts ingested* 25. viii. 25.

	Dates												
		Aug.–Sept. 1925											
Examinations	1906–1925	25	26	27	28	29	30	31	1	2	3	4	5	1925–1933
Microscopic	−	−	−	−	−	−	−	−	−	−	−	−	+	+
Cultural	−	−	−	−	−	−	−	−	−	−	−	+	+	+

This experiment proves not only that the *Endolimax* of *M. sinicus* is transmissible to man, but also that (in my own case, at least) it is non-patho-

[1] The very rare occasions on which I have failed, since infection, to demonstrate *Endolimax* in my stools by cultural methods, are numerically negligible. They almost all occurred at times when I was attempting to infect myself with other protozoa, and used media or methods specially suited to these other organisms. Had I concentrated my attention at such times upon recovering *Endolimax* from primary "negative" cultures, I should doubtless have succeeded on most occasions. The few "negative" cultures recorded were all incompletely studied initial cultures of this sort, and can therefore be discounted. The important point is that, at the time of writing, I am still demonstrably infected with *Endolimax* but with no other intestinal amoeba.

genic. At the time when I infected myself I kept a close watch for any clinical symptoms which might become manifest as a result of the experiment[1]. But nothing happened: I continued to lead my usual life—as regards diet, habits, etc.—and remained normal throughout. No illness, or even indisposition, referable to the infection, has befallen me. If I had not studied my intestinal fauna in such detail I should not have been conscious that it had been augmented.

I may add that *Endolimax* is generally present in my stools in only small numbers. Both amoebae and cysts are often hard to find by direct microscopic examination alone—though usually discoverable by careful search—but the infection is almost invariably demonstrable by suitable cultural methods. I infer that it is a small one, therefore: yet it is quite indistinguishable from a natural human infection. Any competent protozoologist, if he were to subject me to careful examination, would have no hestitation in pronouncing me infected with *Endolimax nana*—the typical human species. My amoebae and cysts are morphologically identical with those which I have found in hundreds of other human beings naturally infected with this organism.

Although I have cultivated *Endolimax* from my stools on innumerable occasions during the last 8 years, I have always found great difficulty in isolating it in "pure" culture. Primary cultures are always heavily contaminated with *Blastocystis* and starch-splitting bacteria, which are extremely troublesome and hard to eradicate. However, I have succeeded in obtaining pure cultures—free from all other protozoa and *Blastocystis*, and capable of continuation *ad libitum*—at least half-a-dozen times: and several such strains I have studied in detail. One of the best ($\Delta 3$) was isolated on 24 September 1925 (a month after experimental infection with Strain Σ) and was voluntarily abandoned—after thorough study of over 80 subcultures—at its 20th generation on 30 October 1925; but as it has already been described[2], in its essential features, I need say no more about it here. All the other strains were similar, and the amoebae and cysts in all of them differed in no way from those of the parent strain (Σ). They served to confirm my earlier observations, but furnished no new information of any importance.

(2) *Transmission from Man to* M. rhesus.

It is very difficult to make any experiments on the transmissibility of *Endolimax* from one species of macaque to another; because most of these monkeys are already naturally infected, so that "clean" animals—demonstrably free from infection—are rarities. I have therefore been able to make as yet only one experiment in which a macaque was used as the subject.

[1] This was all the more necessary because I swallowed not only *Endolimax* cysts—of whose potentialities I had no apprehension—but also a variety of concomitant intestinal bacteria derived from the same simian host. Concerning the pathogenic properties of these I was in complete ignorance.

[2] See Dobell and Laidlaw (1926 *a*), pp. 288, 298, 308: and cf. also p. 443 *supra*.

I had two objects in performing this experiment. I wished, first, to ascertain whether the *Endolimax* of *M. sinicus* could infect *M. rhesus*—which usually harbours a morphologically indistinguishable species: and I wanted also to ascertain whether the *Endolimax* of *M. sinicus*, with which I had infected a man experimentally, could be transmitted back from man to macaque by the ordinary route. I tried to answer both these questions by a single experiment performed with the only macaque which I have yet found naturally uninfected with *Endolimax*. As the evidence for the original non-infection of this monkey is of vital importance, I must give it in detail.

The experimental animal was my tame monkey Jacko, who has already been mentioned more than once[1]. He was a healthy young male *M. rhesus* who came into my possession in 1924. During this year I examined his faeces frequently, and found that he was naturally infected with *E. histolytica* (2 strains at least, a large and a small) but with no other species of amoeba—so far as I could determine. For about a year, however, he was in frequent contact with four of my other monkeys (all *M. sinicus*), with whom he was allowed to play daily: and as all of these were naturally infected with *Endolimax*, I expected that—even though possibly uninfected himself originally—he would show a natural or acquired infection sooner or later. But I was mistaken: for no amoebae or cysts of *Endolimax* ever appeared in his faeces, and I failed consistently to recover this organism in cultures made from them.

At the beginning of August 1925, I resolved to use Jacko for experiment (if possible), and isolated him—in a specially constructed double cage—from all my other monkeys, in order to study him more intensively under conditions which would preclude all chance of accidental infection.

Before Jacko was isolated I had examined his faeces microscopically for about a year. Between 7 August 1924 and 19 August 1925 I have recorded the results of 29 detailed examinations[2], in all of which I failed to find *Endolimax*. During this period I also made cultures from his faeces, at intervals, with similarly negative results: but as these were all made with the object of isolating *E. histolytica* from him, and as I learnt how to cultivate *Endolimax* only in July 1925, I attach less importance to them than to those I made later. The direct microscopic examinations were made very thoroughly, however, and I felt confident that Jacko was uninfected with *Endolimax* when I isolated him for the present experiment.

After I had separated him from his companions, I kept Jacko for a month in the strictest isolation. He was housed in the same room with my other monkeys, but never allowed in contact with them or with their excreta. This was very difficult, but by continual personal solicitude I managed to keep him healthy and happy despite his incarceration. From 19 August until 19 September

[1] See especially Dobell with Bishop (1929) and Dobell (1931).

[2] Actually I examined his faeces much more frequently: but as I did not always record negative examinations (being at that date chiefly on the lookout for monkeys infected with *E. histolytica*), I cannot give more precise figures.

1925—while he was thus completely isolated—I re-examined Jacko's faeces microscopically on 14 different days; and on 9 of these occasions I made every attempt to detect *Endolimax* in them by cultural methods. The examinations were often extremely tedious and laborious, owing to the frequent presence of amoebae (a small strain of *E. histolytica*) of similar size and general appearance. It was thus often necessary to study cultures and subcultures in great detail, and examine stained preparations exhaustively in order to exclude any possible individuals of *Endolimax* from among them. As I was simultaneously engaged in studying my own experimental infection with *Endolimax* in similar detail, daily examinations were impossible at this period: nevertheless, I had satisfied myself that Jacko was still uninfected by 19 September 1925—the date of the present experiment. Up to this time I had examined his faeces microscopically on 43 recorded occasions during more than a year, and had vainly attempted to recover *Endolimax* from them culturally more than a dozen times—the last 14 microscopic and 9 cultural attempts being made very thoroughly under rigidly controlled conditions. The evidence that he was uninfected is therefore strong.

The experiment was performed on 19 September 1925. On this day I fed Jacko with cysts of *Endolimax* from my own stool passed on September 17. They were found to be fairly numerous on that date: so I washed and concentrated them (by centrifuging in sterile distilled water) and then mixed them with a little milk to make them palatable. Jacko—who had been previously well trained for such experiments—readily swallowed the milk and cysts from a pipette put into his mouth. At the time of ingestion the cysts were 54 hours old from the time when they were passed, and had been kept at room-temperature during this period.

As I had already experienced great difficulty in identifying all the small amoebae in Jacko's faeces by the study of cultures—as just noted above—I resolved to determine the result of this experiment by direct microscopic investigation alone: for the cysts of *Endolimax*, when mixed with those of a small strain of *E. histolytica*, are readily identifiable—especially in fixed and stained preparations—and the amoebae can be diagnosed as easily by the same means in fresh faeces as in cultures. So I made no cultures, at first, from Jacko's faeces after his inoculation, but relied upon direct daily microscopic observation of his droppings—confirmed, when necessary, by study of fixed and stained preparations made from them.

The daily examinations of Jacko's faeces remained negative for *Endolimax* from September 19 until September 25. But on the 26th (7 days after inoculation) I discovered a few cysts of this organism, and next day a few amoebae as well: and for the next 4 days cysts or amoebae were regularly present in small numbers. These findings were then confirmed by stained permanent preparations and cultures.

Thereafter Jacko remained infected with *Endolimax* for the rest of his life. He was killed on 15 February 1928, and during the intervening period his

faeces were very carefully studied on more than 100 occasions. During this interval, moreover, he was treated with emetine[1]—in an unsuccessful attempt to remove his natural infections with *E. histolytica*—but the treatment had no effect upon his acquired *Endolimax*. This was frequently obtained in cultures made from his faeces, differed in no way from its originals (derived from Man and *M. sinicus*), and persisted unchanged to the day of his death—2½ years, approximately, after the date of experimental infection.

Though I say that Jacko's infection "persisted," I would add the following qualification. After I infected him with *Endolimax* experimentally, and when I felt sure that the infection was established, I allowed him to associate again with the other members of my monkey-family (4 *M. sinicus* and 1 *M. rhesus*)—all of them known to be naturally infected with *Endolimax*. As I have now obtained evidence that infection with this amoeba can die out spontaneously in *M. rhesus*[2], it is just possible that Jacko's "persistent" infection was not really a continuous infection with the strain inoculated. This may conceivably have died out, and he may then have acquired a reinfection from his companions. He may even have lost his original infection from time to time, and reacquired it by ingesting cysts present in his own faeces. All macaques are excessively dirty in their habits, and such a contingency is impossible to disprove or control. Similar objections can be made against all such experiments, so I can only assert that Jacko was apparently uninfected with *Endolimax* for over a year: that he then acquired an infection, when fed on infective material: and that he remained infected, whilst in contact with his own faeces and with other known infected monkeys, till he was killed 2½ years later.

Jacko's experimental infection with *Endolimax* was in every way exactly like any natural infection which I have studied in *M. rhesus* and *M. sinicus*. The amoebae and cysts found in his faeces, or recoverable from them in culture, were also indistinguishable from a typical strain of *E. nana* in Man. This monkey remained normal and vigorous—apart from wounds received in fighting[3]—until he was killed: and at the necropsy I could find no lesions of any sort in his intestine or elsewhere. After inoculation he continued to grow normally, and on the day of his death—when he was probably between 5 and 6 years old—he weighed 15 kg. (a respectable weight for a captive macaque).

The foregoing experiment proved, to my own satisfaction, that the *Endolimax* of *M. sinicus* can live equally well in *M. rhesus* (even after passage through Man), and furnished further evidence of the identity of the species found

[1] See Dobell with Bishop (1929).

[2] See p. 460 *infra*.

[3] Jacko was terribly injured in a battle royal with the other adult male (Bonar, *M. sinicus*) in my monkey-family on 7. ix. 27: but he recovered, with careful nursing, and his extensive wounds healed almost without a scar.—When he became adult, Jacko was a most powerful and savage animal; and I had to chloroform him because he was so unmanageable and such a menace to all my other monkeys and their attendant. To the end, however, I could do anything I liked with him —except control his fierceness towards others. I sacrificed him with great regret, after four years' close acquaintance.

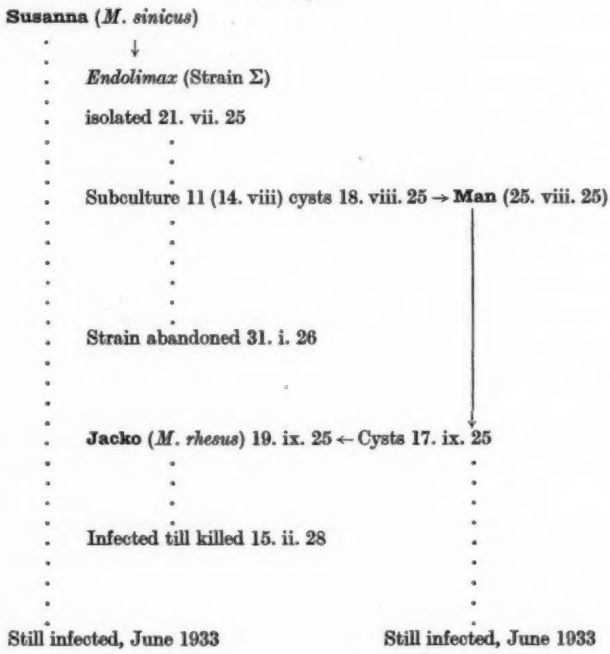
naturally in these various hosts. The experiment is briefly summarized in Table IV.

Table IV. *Infection of* M. rhesus *by means of* Endolimax *cysts* (*Strain derived from* M. sinicus) *out of Man. Cysts ingested* 19. ix. 25.

Dates										
		September 1925								
	7. viii. 24–16. ix. 25	19	20	21	22	23	24	25	26	27. ix. 25–15. ii. 28
Examinations	–	–	–	–	–	–	–	–	+	+

In order to make the whole story described in this and the preceding section readily comprehensible, I also add a scheme showing the principal events graphically.

Scheme.


Susanna (M. sinicus)
Endolimax (Strain Σ)
isolated 21. vii. 25
Subculture 11 (14. viii) cysts 18. viii. 25 → Man (25. viii. 25)
Strain abandoned 31. i. 26
Jacko (M. rhesus) 19. ix. 25 ← Cysts 17. ix. 25
Infected till killed 15. ii. 28
Still infected, June 1933
Still infected, June 1933

(3) *Attempts to infect kittens.*

As far as I am aware no experiments made with the aim of infecting kittens with *Endolimax* have yet been recorded. Nevertheless, it is certain that such experiments have already been performed with *E. nana* unintentionally. This amoeba occurs so commonly in Man, that more than one worker who has tried to infect kittens with *E. histolytica* by administering human faeces (containing its cysts or active forms) must unknowingly have made the experiment—

apparently with negative results. As an instance I may cite the work which I did with Dale in 1916 (Dale and Dobell, 1917). We then made many unsuccessful attempts to infect kittens by feeding them upon cysts of *E. histolytica* in human stools, and several of these—as I now know—contained cysts of *E. nana* also[1]: but no amoebic infection of any sort resulted. Some of the experiments, therefore, indicated—though I did not realize it at the time—that kittens are not infectible with *E. nana*.

In the present series of experiments with monkeys I have made—and recorded partially—some similar observations. In an earlier instalment (Dobell, 1931) I have described attempts to infect kittens with *E. histolytica* derived from the faeces of various Macaques: and some of the monkeys whose faeces were used I knew to be infected also with *Endolimax*. My Kittens 1, 9, and 10, fed with *E. histolytica* cysts in the faeces of my monkey Susanna (*M. sinicus*) in 1924, were also fed with cysts of her *Endolimax* unavoidably present in the same material: and Kittens 3 and 4, inoculated *per anum* with faeces from my monkey Polo (*M. sinicus*) likewise received his *Endolimax* as well as his *E. histolytica*. (See Dobell, 1931; Table II, p. 16.) At a later date (1926), Kitten 28—who acquired a transient infection with *E. histolytica* derived from my monkey Bonar (*M. sinicus*)—was inoculated unsuccessfully with *Endolimax* in the same experiment. (See Dobell, 1928; p. 368: and Dobell, 1931; p. 26.) All these experiments (made primarily to study *E. histolytica*) were therefore simultaneous attempts—though not previously recorded as such—to infect kittens with the *Endolimax* of *M. sinicus*. All the animals concerned were examined with great care, and the final results showed that the 6 kittens inoculated (Nos. 1, 3, 4, 9, 10, 28) were not infected with the *Endolimax* present in the faeces of the 3 individuals of *M. sinicus* used for the experiments —whether injected with amoebae *per anum* (2 kittens), or fed with cysts *per os* (4 kittens).

Most of the foregoing unsuccessful attempts to infect kittens with *Endolimax* from Macaques were made before I had succeeded in cultivating this amoeba. But when I had obtained an excellent strain (Σ) in pure culture, in 1925, I thought it worth while to repeat them—since no such experiment had been made previously. I therefore inoculated 2 fresh kittens (Nos. 22 and 23) with pure cultures of Strain Σ. Neither kitten was used for any other experiment, and their histories are briefly as follows:

Kitten 22. Female, about 7 weeks old. Inoculated[2] *per anum* 21. viii. 25 with all the amoebae in a rich 3-day culture of Strain Σ (12th serial subculture, in "Ehs" medium). She never showed any symptoms referable to the inoculation—such as diarrhoea or dysentery—but remained perfectly normal,

[1] In 1916, when these experiments were made, I was already familiar with the cysts of *E. nana*, though I did not know what they were. They had not then been identified, though their frequent occurrence in samples of human faeces was recognized, and generally recorded in my routine examinations.

[2] Details of the technique employed have been recorded elsewhere (Dobell, 1931; p. 12 *et seq.*).

passing solid faeces (at infrequent intervals) in which no amoebae or cysts were ever discoverable microscopically. On 1. xi. 25 (10 weeks after inoculation), when I had made 7 very thorough negative examinations by cultural methods also, I was satisfied that she was uninfected with *Endolimax*; so I took her home and kept her as a pet, in order to make sure that she would remain normal.

This negative experiment was a parallel to the positive experiment—already recorded—which I made at the same time upon myself. It was conducted with equal care and similar precautions, and its result appeared just as conclusive. I myself acquired an infection with the *Endolimax* of *M. sinicus*, but Kitten 22 did not.

Kitten 22 grew into a beautiful little cat (named "Tabitha"), and remained normal in every way for 2 years. She was then (August 1927) suddenly seized with violent diarrhoea and vomiting, so I took her back to my animal-room in order to nurse and study her. In spite of all my efforts to cure her, however, she got steadily worse, and died in the night of 1–2 November 1927. During the last 2 months of her life I examined her faeces repeatedly—both microscopically and culturally—but I could find no protozoa of any sort, and no worms. At necropsy, I found an extensive enteritis and colitis; and I believed—though I could obtain no conclusive proof—that she had been wilfully poisoned. In any case, her death was not due to my experiment, which was followed up with negative results for 2 years and 2 months.

Kitten 23. Male, about 6 weeks old. Inoculated intrarectally with *Endolimax* (Strain Σ, serial subculture 10; 3 days old, containing abundant amoebae) at the same time as No. 22, and with like precautions.

The result was again completely negative. Microscopic faecal examinations revealed no sign of infection with *Endolimax*, and cultures made on 5 different days during the next 7 weeks were all negative for amoebae, though a heavy infestation with ascarids was found. On 12 October 1925 this kitten, which had been ailing—though not definitely ill—for several days, was unexpectedly found dead in its cage. The *post-mortem* examination revealed no protozoal infection of any sort, but abundant ascarids packed in the duodenum and upper part of the small intestine, where an enteritis was also visible. I attributed the kitten's death to this natural infection with worms, as I could find no other probable cause.

It will thus be evident that I have hitherto failed to infect any kittens experimentally with *Endolimax nana* from Man, or with the closely similar (? identical) amoeba living in Macaques. All the evidence which I have yet obtained indicates that cats are incapable of infection with these organisms, and I can find no indications to the contrary in the literature.

(4) *The duration of infections.*

I will here add a few notes on the persistence of infections with *Endolimax* in Man and Macaques—derived from numerous observations, and records kept for many years.

In the first place, I note that I have never yet seen any infection with *E. nana* die out spontaneously in any human being. I have studied the infections of many individuals for several weeks, and of several for many months: and in a few cases I have been able to follow them for some years. All the subjects have remained infected during the entire period of observation.

The same is true for most of the macaques which I have kept under close observation. In almost all of them, their natural infections with *Endolimax* have persisted unchanged as long as they have been in my possession. There is only one exception to this general rule: my monkey Rosa (*M. rhesus* ♀) has, to my surprise, apparently lost her natural infection completely in the course of the present year (1933). From the time when she first came under observation[1], towards the end of 1926, until February 1933, her infection with *Endolimax* was always easily demonstrable—microscopically and culturally. During this period, moreover, she was three times treated with emetine to eradicate her infections with *E. histolytica*—the last treatment ending on 25 November 1929[2]. Since then she has remained normal and healthy, and no drugs have been administered to her.

I last found *Endolimax* in Rosa's faeces on 15 February 1933: and since then I have examined them over 80 times, both microscopically and culturally on every occasion, until the date of writing (June 1933). During this period of about 16 weeks she has been consistently negative for *Endolimax*, and I am therefore persuaded that her infection has died out spontaneously.

To avoid repetition, and to summarize all my observations (partly recorded elsewhere) on the animals used in the present series of experiments, I present the chief data in tabular form. (See Table V, p. 461.)

III. Discussion.

Before attempting to colligate the data recorded in the foregoing sections, I must recall a few relevant observations made by other workers.

The first "*Endolimax*" reported from any monkey of the genus *Macacus* [= *Macaca* = *Silenus*] was apparently "*Endolimax kueneni*," described very briefly and incompletely by Brug (1921) from *M. cynomolgus* [= *irus*]. As I have pointed out elsewhere, this amoeba is not an *Endolimax* at all, but an

[1] The earliest examinations of this animal were made by Miss Ann Bishop, whose findings I checked personally.

[2] See Dobell with Bishop (1929), and Dobell (1931).

Iodamoeba[1]. At the time when it was first described, however, Brug regarded these genera as synonymous: but there can now be no doubt that he was mistaken, and that "*Endolimax*" *kueneni* should be called *Iodamoeba kueneni*. The type species of *Endolimax* is *E. nana*, and *I. kueneni*—as described by Brug—bears no resemblance to this organism. It is closely similar to—perhaps identical with—*Iodamoeba bütschlii*, the type of *Iodamoeba*.

As noted earlier, however, Brug (1923) subsequently described and named an *Endolimax cynomolgi* from the same simian host in Java. This was a true *Endolimax*, and the first to be recorded from any macaque—so far as I am able to ascertain. I discovered a similar amoeba in *M. sinicus* and *M. rhesus* in the following year (1924).

Table V. *Duration of Infections with the* Endolimax *of Macaques.*

Host	Observed Duration	Remarks
Polo (*M. sinicus*)	August 1924–July 1928 (4 years)	Killed 28. vii. 28 Infected till death
Jacko (*M. rhesus*)	September 1925–February 1928 (nearly 2½ years)	Killed 15. ii. 28 *Experimental infection* Infected till death
Mungo (*M. sinicus*)	August 1924–August 1928 (4 years)	Killed 8. viii. 28 Infected till death
Susanna (*M. sinicus*)	July 1924–June 1933 (9 years)	Still alive and infected
Rosa (*M. rhesus*)	November 1926–February 1933 (6¼ years)	Still alive *Uninfected* for last 4 months
Bonar (*M. sinicus*)	August 1924–September 1927 (3 years)	Killed 8. ix. 27 Infected till death
Samson (*M. rhesus*)	—	Killed 29. x. 24 Infection discovered at necropsy
Delilah (*M. rhesus*)	August 1924–January 1925 (5 months)	Killed 27. i. 25 Infected till death
No. 9 (*M. rhesus*)	—	Died 5. v. 25 Infection discovered at necropsy
No. 10 (*M. nemestrinus*)	—	Killed 3. viii. 27 Infection discovered at necropsy
Man	August 1925–June 1933 (nearly 8 years)	*Experimental infection* still persistent

Some 3 years later, Deschiens (1927) published a few observations made on the intestinal protozoa which he found in divers monkeys kept at the Pasteur Institute (Paris). Among the forms mentioned is "*Endolimax*," which he found in Chimpanzees, Cercopitheques, and *Macacus sinicus*; and with this amoeba—or these amoebae, for it has never been proved that they are all of one species—he performed various experiments. He states definitely, however, that his "*Endolimax*" were of the "type" of "*E. kueneni*"—"*à kystes iodophiles et uninucléés*"—and they were therefore *Iodamoeba* and not *Endolimax*. (Curiously enough, Deschiens appears to have found no true *Endolimax* in any of his

[1] See Dobell and O'Connor (1921; p. 57 note). The full synonymy—to date—of *Endolimax* and *Iodamoeba* will be found in my monograph of 1919, to which there is still little worth adding as regards the nomenclature of these genera.

monkeys.) Consequently, these observations and experiments of Deschiens do not now concern us, and there appears to be no justification whatsoever for supposing—with Hegner and Chu (1930)—that they relate to *Endolimax nana*. The words of Deschiens himself warrant no such inference.

More detailed study of a true *Endolimax* from monkeys has been made by Kessel (1928), who examined 20 Macaques in China and found 18 of them infected with "*E. nana*." According to his published data[1] he discovered this species of amoeba in 4 specimens of *Macacus irus* [= *cynomolgus*], 2 *M. sancti-johannis*, 4 *M. lasiotis*, and 8 *M. rhesus*. Only 1 individual of *M. lasiotis* and 1 *M. irus* in his series appeared to be uninfected[2].

If the findings recorded by Brug and Kessel be added to my own, then it must be concluded that an *Endolimax* morphologically indistinguishable from *E. nana* of Man occurs with great frequency in captive monkeys of the genus *Macacus*. The only record of the occurrence of a similar organism in wild macaques has been more recently registered by Hegner and Chu (1930), who discovered "*E. nana*"—specifically identified on very questionable statistical grounds—in 22 out of 44 dead individuals of *M. philippinensis*[3]. More careful study of these animals by cultural methods would probably have revealed a much higher incidence of infection.

Though most of the published records of intestinal protozoa from monkeys are glaringly defective, and some of them are doubtful in the extreme, it seems likely that an *Endolimax* closely similar to *E. nana* (of Man) occurs with great frequency in many of the Macaques. Some such organism has already been described—more or less—from at least 7 species of *Macacus*, and possibly from others. I epitomize the probably accurate records, with their recorders, in Table VI: but I do not claim that it is complete. Owing to the ambiguity of many descriptions, and the difficulty of finding and understanding all that has been published, I still possess incomplete information on this subject.

Table VI. *Occurrence of* Endolimax *in species of* Macacus [= Macaca = Silenus].

Host	*Endolimax* recorded by
(1) *M. cynomolgus* [=*irus*]	Brug (1923), Kessel (1928)
(2) *M. sinicus* [=*radiatus*]	Dobell (1924)[4]
(3) *M. rhesus* [=*mulatta*]	Dobell (1924)[4], Kessel (1928)
(4) *M. sancti-johannis*	Kessel (1928)
(5) *M. lasiotis*	Kessel (1928)
(6) *M. nemestrinus*	Dobell (1928)
(7) *M. philippinensis*	Hegner and Chu (1930)

[1] Kessel (1928); Table 1, p. 280.

[2] The latter (No. 6) was "examined three times a week from January 2 to March 8, 1924": but I can find no record of the actual examinations made on the "uninfected" *M. lasiotis* (No. 17). No mention is made of cultures from these animals.

[3] "The species common on the Island of Luzon, one of the Philippine Islands, the scientific name of which [!] is usually given as *Macacus philippinensis*" (Hegner and Chu, 1930; p. 62).

[4] See *Ann. Rept. of Med. Res. Council* 1924–25 and 1925–26: also Dobell and Laidlaw (1926, 1926 *a*), and Dobell (1928, 1931).

It is a somewhat curious fact that almost all recent writers who have recorded any *Endolimax* from any species of *Macacus* have unhesitatingly identified the amoebae which they found as "*E. nana*." Without any other evidence[1] than superficial morphological resemblance at certain stages of development, they have incontinently jumped to the conclusion that only one species of *Endolimax* inhabits all the Macaques, and that this "species" is identical with that known to live in Man. Yet so far as I am aware nobody has hitherto even tried to show that the *Endolimax* found in any one species of *Macacus* is transmissible to another; and nobody has yet studied and described a single one of these amoebae at all stages in its life-cycle. Current conclusions, consequently, regarding all these organisms, seem—to say the least—somewhat ill-founded.

Although no attempt has previously been made to infect one species of *Macacus* experimentally with the *Endolimax* proper to another species, some experiments have been recorded by Kessel (1928) to prove that *E. nana* (of Man) is transmissible to certain Macaques. His work must therefore be considered here. Unfortunately his data relating to *Endolimax* are not fully recorded; and, such as they are, are scattered through his text or incorporated in general tables. But the following information can be extracted from his paper:

Kessel (1928), working in Peking with monkeys of unknown origin obtained from dealers, found one individual (No. 6, *Macacus irus*) uninfected with any species of *Endolimax*[2]. His evidence for its non-infection rested on the negative results of examinations made "three times a week from January 2 to March 8, 1924[3]": that is, about 28 times during a period of some 9 weeks. This is strong evidence of non-infection—assuming that the examiner was thoroughly trustworthy and competent. On an unrecorded later date this macaque (No. 6) was fed[4] on "*E. nana*, *Iodamoeba*, and *Giardia*" in human faeces—whether free forms or cysts is not stated—and in "the routine follow-up examinations... made from three to six weeks after the feeding," *E. nana* was "found to have been established[5]." No other evidence of establishment, or for the success of this experiment, is presented. Such sketchily recorded data are not altogether convincing, but if we accept them unreservedly at their face-value they indicate that *E. nana* can inhabit *M. irus*—at least for a short time.

Kessel also records another experiment, made with *E. nana* and a *Macacus rhesus*[6]. This monkey (his No. 14) was found to be naturally infected with

[1] Some evidence has been available to recent workers, however, in my own papers preliminary to the present report. But as these have been generally disregarded—or, at least, not mentioned—by previous authors, I must conclude that their own inferences have been drawn independently.

[2] Kessel (1928), Table 1, p. 280; Table 4, p. 294.

[3] Kessel (1928), p. 295. It is not stated *how* these examinations were made, or by whom: but presumably no cultures were studied, as nobody at that date knew how to cultivate *Endolimax*.

[4] Kessel (1928), Table 4 [not 5, as stated by himself, p. 293], p. 294.

[5] Kessel (1928), p. 295. As this author records his data so casually, he can hardly complain if I attach too little weight to them.

[6] See Kessel (1928); Tables 1 and 4, and p. 295.

"*E. nana*." It was then treated with "yatren," in unspecified dosage for an unrecorded time, and then found to have been "cured" of its infection. The evidence for this rests upon the fact that it was "examined [? how] twenty-eight times between January 28 and April 20, 1925, following the treatment" with consistently negative results. If all these examinations were made microscopically and culturally, by a person[1] familiar with all the protozoa of macaques, this is good evidence of non-infection: else it is not. On 21 April 1925 the monkey was fed on human faeces containing *Entamoeba histolytica*, *E. coli*, *Endolimax nana*, and *Iodamoeba bütschlii* [what stages—whether amoebae or cysts—is not stated], and "cysts [no species recorded] first appeared in the stool of monkey No. 14 on May 2, twelve [eleven, according to my reckoning] days after the first feeding. Subsequent examination of the feces at intervals until July 6 showed that...*E. nana* had been established in monkey no. 14." The way in which this "blunderbuss" experiment was made and recorded is obviously unsatisfactory and unconvincing: but if one of its apparent results be accepted as correct, it indicates that *E. nana* (of Man) can infect *M. rhesus*.

These two experiments of Kessel are of considerable interest in the present connexion: for they show, seemingly, that *E. nana* is transmissible to *M. irus* and *M. rhesus*. I fully believe that this conclusion is correct, but I greatly regret that my American co-worker has furnished such inconclusive evidence for our belief.

If the results of Kessel's experiments be accepted, and if they be added to my own (recorded in the present paper), then our knowledge of *Endolimax*—from Man and Macaques—amounts to this: (1) Man is naturally infected with a species (*E. nana*) indistinguishable morphologically from similar forms living in various Macaques; (2) *E. nana* itself has been experimentally implanted into *Macacus irus* and *M. rhesus*; and (3) the *Endolimax* of *M. sinicus* has been experimentally implanted into Man, and from Man into *M. rhesus*. In all these hosts the infections—natural and experimental—appear alike in every way; and it therefore seems legitimate to conclude that the *Endolimax* naturally occurring in Man (*E. nana*) is specifically identical with that found in *M. sinicus*, *M. rhesus*, and *M. irus* [= *cynomolgus*].

It follows from this that "*Endolimax cynomolgi* Brug, 1923" is a synonym of *E. nana*[2]; and the separate simian "species" must consequently be abolished. If we are content with morphological evidence—unsupported by careful experiment—we may even infer that at least 7 different species of *Macacus* harbour *E. nana*: and bolder speculators might even say, with much plausi-

[1] At the risk of appearing pedantic I must once more emphasize the paramount importance of exact information in all published records of this sort. In such an experiment, where detailed accuracy means everything, it makes all the difference in the world, for the interpretation, if one knows whether the worker himself made the negative examinations, or whether they rest upon the reports of unknown Chinese laboratory assistants.

[2] *Endolimax nana* (Wenyon & O'Connor, 1917) Brug, 1918: cf. Dobell (1919), p. 101 *et seq.*

bility, that this amoeba is a common intestinal inhabitant of Man and the Macaques generally.

Beyond this point it is unsafe to push our speculations at the moment: and I would repeat once more that it is, in my opinion, unscientific to assume that all "monkeys" are equivalent and all their intestinal protozoa identical. When Kessel says[1] that I "have no hesitation in assigning the *Endolimax* of man and of the monkey to the same species," he unintentionally misrepresents my views entirely. Of "the monkey" I know nothing, nor of its intestinal protozoa: the term means as little to me as "the rodent" or "the carnivore," and I hold it to be a gross error—from the standpoint of parasitology—thus to confound all the multifarious members in any large Class of mammals. My own belief, based on all the evidence known to me, is that *Endolimax nana* naturally inhabits not only Man but also *Macacus sinicus*, *M. rhesus*, *M. nemestrinus*, *M. cynomolgus*, and probably other monkeys of the same genus. About other Primates I know nothing, in this connexion, and therefore preserve an open mind.

For such conclusions there is firm foundation, but to go further is still unwarranted by any published observation or experiment. In the absence of exact information and evidence, all wider generalizations are at present unjustifiable and premature: and their proof—or disproof—will certainly require many years of work far more careful and critical than that previously reported by most students of this very complex subject.

And even when we know all that can be known about the *Endolimax* of the Macaques and Man, there are still some pretty problems connected with the occurrence of closely similar—perhaps identical—amoebae in domesticated animals. Some years ago Krediet (1921)[2] recorded his discovery of "*Endolimax nana* (?)" in Dutch oxen, swine, and sheep. Four years later Chiang (1925) described an amoeba ("*Endolimax ratti*"), seemingly indistinguishable from *E. nana*, from laboratory rats. Although Kessel (1928 *a*) and Frye and Meleney (1932) have since studied "*E. nana*" from domestic pigs, we still know very little about any of these organisms. Kessel, however, describes some experiments which indicate that *E. nana* is transmissible from men to pigs, and future work may possibly show that this "amoeba of man" really lives in a variety of mammals but distantly related to Man and to one another.

At all events, it is obviously rash, in our present ignorance, to base any phylogenetic speculations[3] about its hosts upon our fragmentary knowledge of the distribution of *Endolimax nana*. On such matters a modern worker may well be content to remain dumb—remembering the words of the ancient sage of Shirâz: "A wise man is, like a vase in a druggist's shop, silent, but full of virtues[4]."

[1] Kessel (1928; p. 287).

[2] This interesting work has been generally overlooked, though I called attention to it and analysed its contents in some detail in *Trop. Dis. Bull.* (1922), xix, 716.

[3] Cf. Hegner (1928).

[4] The *Gulistân* of Sa'di (*ca.* A.D. 1258), viii, § 62: translated by James Ross (1823).

IV. Summary and Conclusions.

Amoebae of the genus *Endolimax* have been found naturally inhabiting the large intestine of 3 species of *Macacus* (*M. sinicus* [= *radiatus*], *M. rhesus* [= *mulatta*], *M. nemestrinus*).

In all these hosts the amoebae are morphologically and culturally identical, at all stages of development, and are indistinguishable by any structural or cultural character from *Endolimax nana* of Man—with which they have been carefully compared. The several forms have been studied in "pure" culture (*i.e.*, free from all other protozoa, but accompanied by bacteria suitable for their growth).

The *Endolimax* of *M. sinicus*, isolated in "pure" culture, has been experimentally transmitted to an uninfected man by the natural route (cysts *per os*): and in this human subject it has produced an infection indistinguishable from a natural one with *E. nana*. The induced infection has now persisted unchanged for close on 8 years.

From the experimentally infected man the amoeba has been artificially transmitted to an uninfected *M. rhesus*, in which it gave rise to an infection—likewise indistinguishable from a natural simian infection—which persisted for the rest of the monkey's life (2½ years).

Some further information is given about the distribution, cultivation, emetine-resistance, and other peculiarities of the forms of *Endolimax* found in Macaques and Man; and the findings of other workers with similar organisms are briefly considered. From all the evidence available it is concluded that *E. nana* naturally lives not only in Man but also in at least 3 different species of *Macacus*, and probably in monkeys of this genus generally. As yet there is no evidence that more than one species of *Endolimax* inhabits any of these hosts.

Infections with *E. nana*, whether in Man or Macaques, have usually been found to endure indefinitely; but a case (*M. rhesus*) has been observed in which the infection appears to have died out spontaneously after persisting for a known period of over 6 years.

Attempts to infect kittens with *E. nana* have all been unsuccessful hitherto, but there is evidence (from the work of others) that this amoeba may inhabit some other domestic mammals. Its host-distribution in nature needs further investigation.

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THE MALLOPHAGAN GENUS *TRICHOPHILOPTERUS*.

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(With 2 Figures in the Text.)

Extraordinary as any of the Mallophaga are when compared with conventional insect forms, there are some species of the order which are still more peculiar than the majority of their fellows. Among these unusually strange forms is that here to be dealt with, a species that seems to combine within itself the characteristics of two otherwise trenchantly separated groups, that possesses striking specific peculiarities and that in addition is decidedly aberrant in its choice of host. It has but once been recorded. The availability of further specimens makes possible additions to our knowledge of its structure and a reconsideration of its relationships.

Genus **TRICHOPHILOPTERUS** Stobbe.

1913. Stobbe, *Entomologische Rundschau*, **30**, 105, 2 figs.
1919. Mjöberg, *Entomologisk Tidskrift*, **40**, 94.
1929. Ewing, *A Manual of External Parasites*, pp. 95, 102.

This genus was established by Stobbe for the single species, *Trichophilopterus babakotophilus* Stobbe, from a lemur, *Lichanotus indri*, of Madagascar. It was assigned by its author to the family Philopteridae and thus constituted the only known member of this family from a mammal. Mjöberg (1919) recorded the discovery of a supposed second species from *Propithecus* sp. and proposed for this the name *T. stobbei*, with the notation that it was "to be described at a later occasion." Apparently this description has never appeared and the name remains a *nomen nudum*. At this same time Mjöberg proposed the new family Trichophilopteridae for this genus, according it a brief and entirely uncritical diagnosis. The family was accepted by Ewing (1929) and separated by him from the Philopteridae by the characterisation: "Last segment of antennae somewhat swollen or clubbed; sides of forehead provided with stout recurved hooks. On mammals." The writer maintains that this family should not be recognised as distinct from the Philopteridae in spite of the peculiarities of its type species. A discussion of the critical points will be presented following a redescription of the species.

Trichophilopterus babakotophilus Stobbe.

(Figs. 1, 2.)

References identical with those for the genus.

Specimens examined. Five males and six females from *Lemur coronatus*, Madagascar, taken by the writer some years ago from skin No. 83961 in the United States National Museum. A pair of these specimens will be deposited in that Museum, the remainder in the Stanford University collection.

Female (Fig. 1). Length 1·3 mm. A robust form with almost rectangular and heavily sclerotic head, which strongly recalls the head form of many

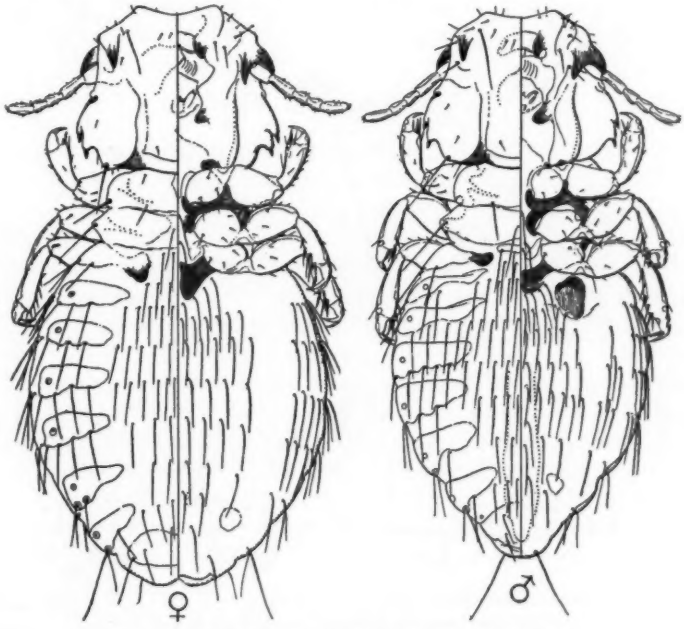

Fig. 1. *Trichophilopterus babakotophilus* Stobbe, ♂ and ♀.

species of Trichodectidae. Head armed with stout, sclerotic hooks, one being on the dorsal side at the anterior-lateral angle and the usual preantennal hook being divided into two, covering the base of the antenna on the ventral side. Sides of the hind head armed with a pair of stout, thorn-like setae. Forehead short and heavily sclerotic, lacking the characteristic markings of most Philopterids. Antennae (Fig. 2 A) five-segmented, slender, the last three segments forming a slight club and faintly squamate-reticulate. Mandibles (Fig. 2 B) very large and heavy, with transverse furrows, fitted for hair-clasping as in the Trichodectidae. Maxilla (Fig. 2 B, *m*) a simple lobe. Labium (Fig. 2 G) of the form characteristic of the Ischnocera. Pharyngeal sclerite present.

Thorax very short, mesonotum entirely suppressed or fused with the metanotum, the latter but little exceeding the pronotum in width and not at all in length. Prothoracic spiracle small. Ventral side with the normal skeletal bars, the coxae crowded and leaving but a narrow sternal area. Anterior legs with the tibio-tarsus (Fig. 2 D) rather stout, two claws present, but one reduced to an exceedingly minute vestige. Middle and posterior

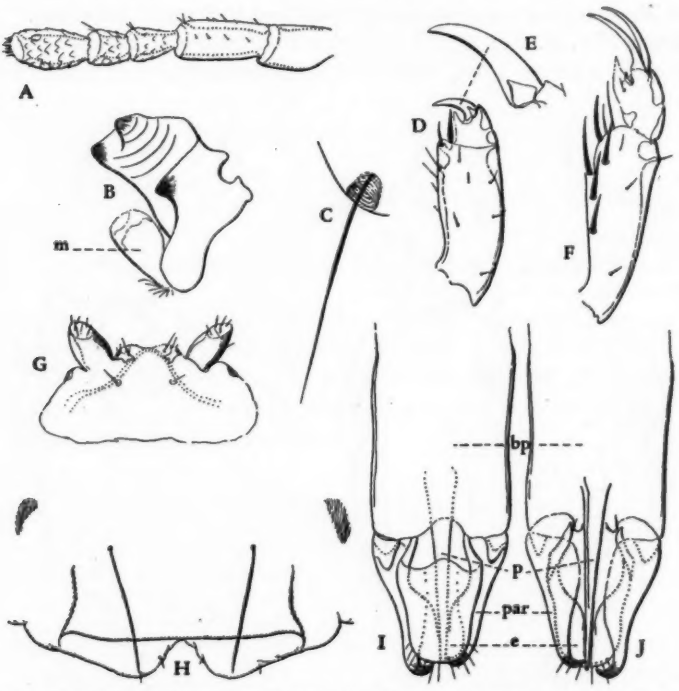

Fig. 2. *Trichophilopterus babakotophilus* Stobbe. A, antenna; B, dorsal aspect of right mandible, with maxilla (*m*); C, sensory seta; D, anterior tibio-tarsus; E, anterior claws; F, posterior tibio-tarsus; G, labium; H, genital region of female; I, ventral aspect of genitalia of male, including only a portion of the basal plate; J, dorsal aspect of the same.

tibio-tarsi (Fig. 2 F) longer and relatively more slender, with two equally developed claws.

Abdomen broadly oval, membranous except for the paratergal plates, the ninth tergite and a conspicuous, heavily sclerotic bar which may be regarded as the first apparent sternite. This latter plate is divided medially and connects with the pleurite of the metathorax, being possibly an extension of the pleurum. First paratergal plate with a quite heavily sclerotic lobe at its mesal extremity. Genital plate entirely lacking, there being only a pair of very small and weakly sclerotic areas in the genital region. Setae arranged in single rows, as indicated in the figure. Gonopophyses entirely lacking. Vulva a simple fold

(Fig. 2 H), which appears close to the apex of the body. Paratergal plates of the seventh segment with two and of the eighth with one sensory seta, these borne in deep, transversely ringed pits (Fig. 2 C). Spiracles quite small and simple, borne in the paratergal plates of the third to eighth segments.

Male (Fig. 1). Length 1·2 mm. In all respects very similar to the female, but with the abdomen pointed. Antennae unmodified. Venter of the abdomen with a pair of widely separated sclerotic areas on what is probably the third sternite. Genital plate lacking, there being but a pair of small, faintly sclerotic areas as in the female. Genitalia (Figs. 2 I, J) with a long and rather slender basal plate (*bp*) to the truncate apex of which articulate the stout, hooked parameres (*par*), these overlying a broad, sclerotic median lobe (*e*) that may be regarded as endomeral; penis (*p*) long and slender.

NOTES ON RELATIONSHIPS.

The trichodectid-like appearance of this species, evident in the form of the head and especially of the mandibles, is at once noticeable but is misleading. The two-clawed tarsi, the form of the paratergal plates and the absence of gonopophyses are definitely non-trichodectid. It may be surmised that the species is either a connecting link between the Philopteridae and the Trichodectidae or that the similarities to the latter family are the result of convergence, the head and mandibles being modified for the grasping of hairs rather than feathers.

The examination of a series of forms belonging to the Philopteridae—as that family is at present understood—indicates strongly that *Trichophilopterus* is merely an extreme member of this group. The slightly clavate antennae differ only in the smallest degree from, for example, the antennae of *Psitticonirmus australis* Harrison. The absence of the usual plates of the forehead—the "signature"—is shared with *Goniodes* and *Goniocotes* and other genera. Compared with such a genus as *Physconella* the hooks on the head lose any special significance. The vestigal claw of the anterior tarsi is connected through a complete series with Philopterids in which both claws are fully developed, the tendency toward a reduction of one of these claws being widespread in this family. The sensory setae of the seventh and eight paratergites are unusual, but seem to be present also in *Psitticonirmus australis*.

The occurrence of the species upon lemurs is indeed peculiar, it being the only two clawed Ischnoceran found upon mammals. But anomalous as this may be, it is not of itself sufficient basis for deciding a moot question of relationship.

It is the writer's conclusion that the family Trichophilopteridae cannot justifiably be maintained and that the genus *Trichophilopterus* should be referred once more to the Philopteridae.

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ARGAS BOUETI, NOUVELLE ESPÈCE D'IXODIDÉ PARASITE DES CHAUVE-SOURIS AU SOUDAN FRANÇAIS.

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(Avec Planche XXVIII, figs. 1–8, et Figs. I–VI dans le texte.)

LES collections de l'Institut Pasteur possèdent un assez grand nombre d'exemplaires, à tous les stades, d'un curieux Argasiné recueilli en 1911 par la mission Bouet-Roubaud, au cours de son exploration parasitologique des régions soudanaises de l'Afrique Occidentale française. Cet Acarien avait été rencontré par G. Bouet à Djenné, dans des trous d'arbres ou des cases obscures servant de refuges à plusieurs espèces de chauve-souris: *Hipposiderus tridens* Et. Geoff., *Taphozoüs perforatus* Geoff., cette dernière espèce parasitée par un trypanosome.

L'examen de ces échantillons montre qu'incontestablement apparenté à l'*Argas vespertilionis* des différentes régions de l'ancien monde par ses caractères généraux, l'Argas des chauve-souris soudanaises s'en distingue au premier

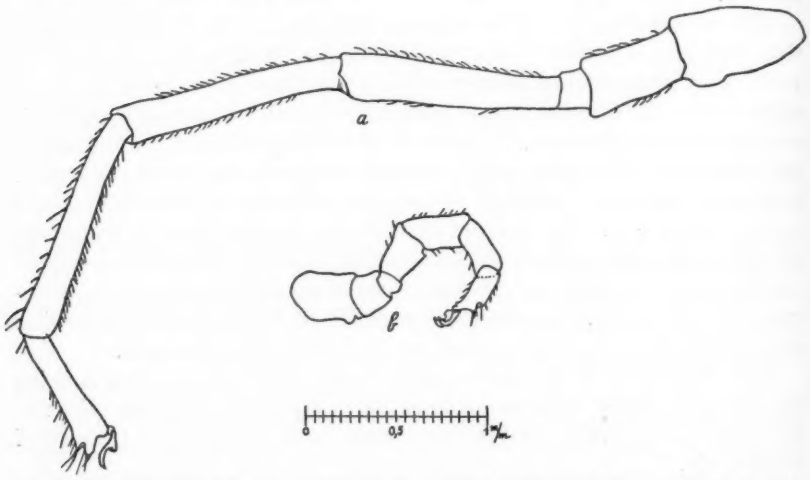


Fig. I. (*a*) *Argas boueti* et (*b*) *A. vespertilionis*. Aspect et dimensions comparées de la patte antérieure.

abord par ses pattes remarquablement longues et grêles (Fig. I), sa forme un peu différente, la disposition toute particulière du rostre et par ses dimensions plus considérables. A vrai dire, si l'on se réfère aux descriptions données pour la

forme adulte de l'*Argas vespertilionis*, par Nuttall et Warburton[1], par Patton et Cragg[2], par E. Borel[3], des divergences notables ne sont pas sans apparaître entre les différents représentants actuellement étudiés de cette espèce, encore relativement peu connue, à tel point qu'il est permis de se demander si plusieurs types spécifiques ou raciaux d'Argasinés parasites spécifiques des chauves-souris ne seraient pas actuellement réunis sous une même appellation. Mais, en ce qui concerne l'Argas soudanais, la légitimité de sa distinction complète d'avec les différents types connus d'*A. vespertilionis* n'est pas contestable. Elle apparaîtra nettement à la description donnée ci-après, ainsi qu'à l'examen de la Pl. (Figs. 1–7) dans laquelle nous donnons comparativement, au même grossissement, des photographies, aimablement prises par M. H. Gaschen, de la nouvelle forme (Figs. 1, 2, 4, 5) et celle d'un adulte de l'*A. vespertilionis* européen (Fig. 8), obtenu directement d'élevage sur souris à partir de la nymphe, par J. Colas-Belcour.

Nous dédions la nouvelle espèce d'Argas au Dr G. Bouet qui l'a découverte au Soudan.

DESCRIPTION D'*ARGAS BOUETI* N.SP.

Forme Adulte (Pl. Figs. 3–5 et Figs. II–IV). **Femelle:** corps subcirculaire postérieurement, s'atténuant en une avancée conique bien caractérisée au tiers antérieur.

La surface dorsale est légèrement et irrégulièrement convexe en son milieu, fortement aplatie sur les bords qui, chez les exemplaires en état d'inanition, se retroussent plus ou moins dorsalement, donnant à l'organisme un aspect foliacé très particulier (Fig. 2).

Tégument dorsal parsemé de *disques* en petits tubercules arrondis, plus clairs au centre, irrégulièrement répartis. En son milieu, chez les individus incomplètement en réplétion, la face dorsale présente de larges dépressions circulaires, en "empreintes de gouttes de pluie" qui s'atténuent progressivement vers les bords en prenant un contour plus ou moins polygonal, irrégulier (Fig. 1). Bordure périphérique formant un mince liséré sans la striation bien marquée de l'espèce courante.

En avant, présence, comme chez *A. vespertilionis*, d'un court capuchon conique, projeté en avant et obliquement vers le bas, d'environ 0·5 mm. de long, convexe en dessus, excavé en dessous, et qui abrite le rostre au repos.

Face ventrale. Pli coxal mince mais bien accusé. Anus ovalaire mesurant chez le specimen étudié 238μ × 125μ, légèrement en arrière de la partie médiane du corps. Les deux valves sont garnies de 9 paires de fortes soies entrecroisées (Fig. IV). En outre, aux deux extrémités antérieure et postérieure, une paire de soies, peu visibles, dirigées plus ou moins dans le sens axial. Le pli supracoxal est large, développé de la base du capuchon à la hanche IV.

[1] *Ticks, a Monograph of the Ixodoidea.* Part I, pp. 34–9 (1908).
[2] *A Text-book of Medical Entomology*, p. 582 (1913).
[3] *Bull. Soc. Path. Exot.* **16**, 328 (18 avril 1928).

La vulve s'ouvre largement au niveau de l'extrémité postérieure de la première paire de hanches. En arrière de l'anus, un peu en avant du tiers de la distance de l'anus au bord postérieur, sont présents, comme chez *A. vespertilionis*, les orifices de l'organe énigmatique (Fig. 4, *O*). Ces orifices, situés de chaque côté de la dépression médiane post-anale, sont en forme de boutonnières ou de fentes étroites, légèrement crescentiformes, à bords lisses et non striés. Ces fentes sont un peu plus longues que le diamètre transversal de l'anus, et s'ouvrent, comme chez *A. vespertilionis*, dans une aire lisse, dépourvue de granulations.

L'aire située entre l'orifice vulvaire et la base du capitulum est couverte de fines stries longitudinales. Les granulations ou disques qui parsèment l'étendue de la face ventrale sont uniformément clairsemées et ne présentent pas la distribution radiaire régulière qui caractérise *A. vespertilionis*.

Le *Capitulum* est profondément rétractile à l'intérieur d'une curieuse gaine cylindrique, épaisse et semi-rigide, d'aspect rugueux, grossièrement godronnée, dont les bords, à l'état de rétraction complète, simulent latéralement deux sortes de *joues* dans le prolongement des bords du capuchon. En activité, le Capitulum exsertile se projette hors de sa gaine (Fig. 6) sur plus de la moitié de sa longueur. Le rostre allongé, dirigé obliquement en avant, quand l'organe est en extension totale peut dépasser le 1/5 de la longueur du corps. La base du capitulum est étroite et allongée, cylindrique.

L'Hypostome étroit, conique, d'environ 100μ de long pour la partie dentée, est pourvu d'une seule rangée de 11 dents, uniquement marginales comme chez *A. vespertilionis*, implantées jusqu'à l'extrémité. Au niveau de la couronne, chez certains exemplaires, on distingue de fines denticules amorçant une deuxième rangée. Présence de deux longues soies post-hypostomales, légèrement incurvées à l'extrémité, plus longues que l'hypostome, atteignant le niveau du 4ème article des palpes (Fig. II).

Palpes: La longueur des articles est en ordre décroissant de 1 à 4. Les trois premiers articles sont revêtus sur le bord dorsal interne (et sur la face interne du 2ème) de plusieurs soies fortes, finement barbelées, incurvées vers l'avant. L'article IV porte à son extrémité digitiforme 5 à 6 soies courtes, spiniformes, et deux internes plus longues (Figs. II et III).

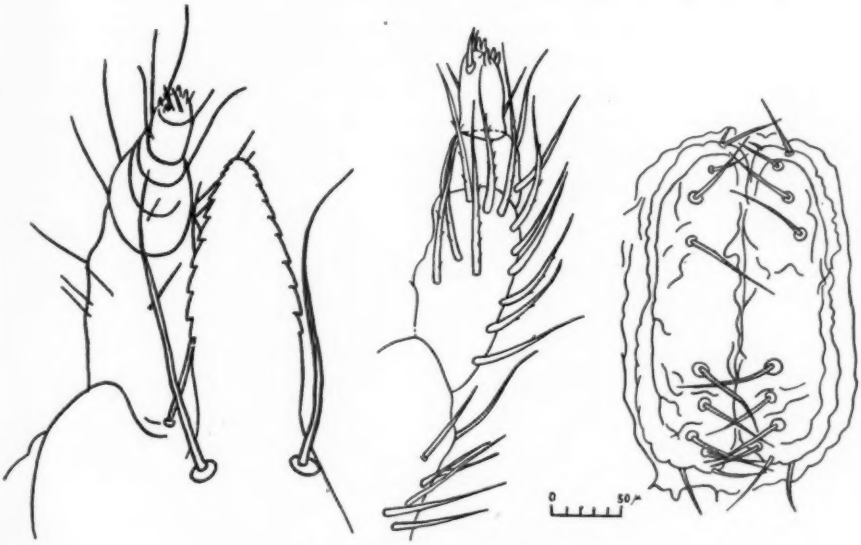
Chélicères à doigts grêles et très allongés, à dents peu distinctes.

Pattes (Fig. I *a*) longues et grêles, surtout la première paire; l'allongement porte surtout sur les fémurs, tibias, protarses et tarses. La hanche I, subconique, est nettement séparée des autres. L'extrémité distale du tarse 1 est tronquée brusquement; la protubérance portant l'organe de Haller est fortement développée et recouvre dorsalement l'armature unguéale. Cette disposition est très différente de celle présentée par l'*A. vespertilionis* (Fig. I *b*). Les tarses des autres paires sont plus ou moins coniques à l'extrémité et portent des griffes beaucoup plus longues et plus fortes que celles de la première paire. La longueur des différentes paires de pattes décroît dans l'ordre: 4ème, 1ère, 3ème, 2ème paires. Pour un exemplaire, par exemple, nous avons mesuré les longueurs

suivantes: 1ère paire, 5·92 mm.; 2ème paire, 5·15 mm.; 3ème paire, 5·75 mm.; 4ème paire, 7·32 mm. Les différentes pattes portent un revêtement uniforme de soies courtes et serrées.

Stigmates situés entre les pattes III et IV, au niveau des trochanters. Le péritrème, en croissant, regarde en arrière et en dedans. Dimensions sur un exemplaire: 175μ de long, largeur 58μ.

Dimensions de la femelle: L'exemplaire monté qui a servi de type à la description mesurait 6·3 mm. de longueur (capuchon compris) × 5·3 mm. Des exemplaires de taille légèrement inférieure mesuraient 5·6 mm. × 4·8 mm.



Figs. II–IV. *Argas boueti*. Adulte.

Fig. II. Palpe et hypostome, vus ventralement.
Fig. III. Palpe vu dorsalement. Fig. IV. Orifice anal (♀).

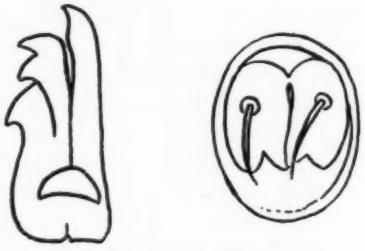
Mâle (Pl. Fig. 5). En dehors des distinctions sexuelles habituelles, les dimensions sont légèrement réduites.

Nymphe (Pl. Fig. 1). Le stade le plus jeune observé mesure 3·5 mm. de long sur 3 mm. de largeur. Téguments finement sculptés, uniformément, en zigzag. La bordure périphérique d'éléments cellulaires est très étroite. Disques peu nombreux, irrégulièrement répartis. La gaine du rostre, épaisse et courte, forme une sorte de capsule dont le tégument montre la même ornementation godronnée que chez l'adulte. *Pattes* longues et grêles, du même type que chez l'adulte. Les coxae de la paire antérieure sont légèrement séparés des autres. Les nymphes au 2ème stade ne se différencient des adultes que par leur taille et l'absence des orifices génitaux. Aux stades nymphaux, chez les plus jeunes observés (3·5 mm.), les fentes de l'organe énigmatique sont déjà visibles.

Larve (Pl. Fig. 7, Figs. V et VI). Corps triangulaire, arrondi, excavé antérieurement en une sorte de cupule d'où émerge le capitulum, en partie visible dorsalement. Cette cupule de la face antérieure représente l'ébauche de la gaine où s'invagine le rostre dans les stades plus âgés.

Les téguments présentent une fine striature transverse, très particulière, s'étendant sensiblement de la région des pattes moyennes jusqu'au voisinage du bord postérieur où elle s'atténue progressivement. Présence de soies courtes, clairsemées, une vingtaine à la face dorsale, surtout bien visibles au bord postérieur, quatre à la face ventrale région anale. L'anus, comme chez la larve d'*A. vespertilionis*, ne présente qu'une paire de soies (Fig. VI).

Pattes effilées, subégales, le tarse nettement plus allongé que les autres articles, courts et quadrangulaires, portant des soies fortes, groupées pour la plupart en verticilles, proches des articulations. Le tarse 1 des larves, à l'encontre des stades précédents, est effilé, conique, dépourvu de protubérance dorsale bien accusée. Hanches très allongées, juxtaposées.

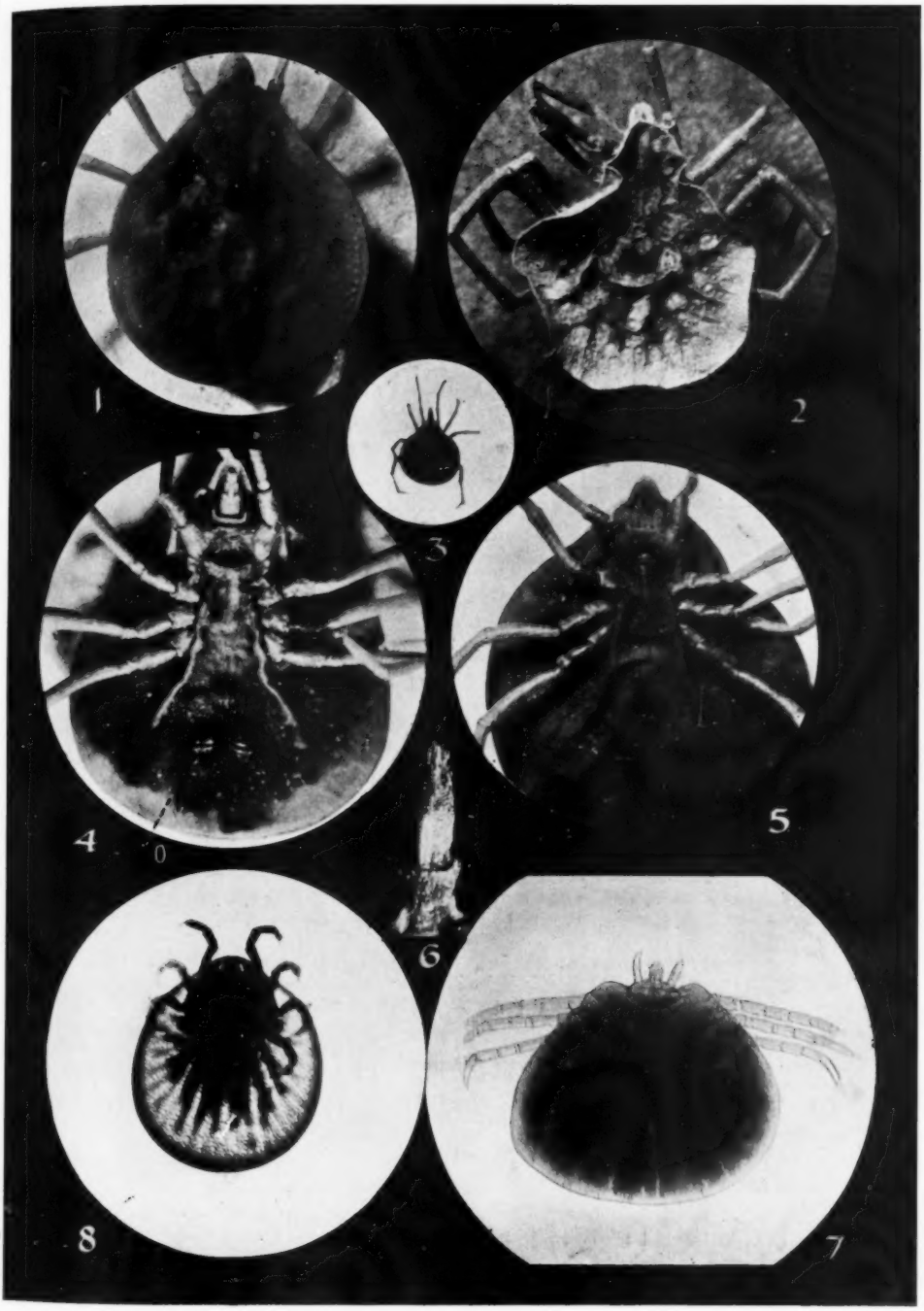


Figs. V et VI. *Argas boueti*. Larve.
Fig. V. Doigts de chélicère gauche.
Fig. VI. Orifice anal.

Capitulum court et aplati, atteignant en largeur environ la moitié de la largeur de la face antérieure du corps, au niveau des bords de la cupule. *Hypostome* à deux rangées de dents bien marquées, une troisième seulement ébauchée sur certains individus. *Chélicères* à doigt interne simple, le doigt externe tridenté. Présence en outre d'une apophyse basilaire (Fig. V) ovaliforme. *Palpes*: articles linéaires, cylindriques, dont les longueurs respectives sont dans l'ordre: $2 > 3 > 4 > 1$.

Habitat. Hôtes. Soudan français, région de Djenné. Semble parasiter plusieurs espèces de chauve-souris, mais plus particulièrement *Taphozoüs perforatus* Geoff. La présence des nymphes de l'Argas a été constatée par G. Bouet sur le corps de certaines chauve-souris de cette espèce qui se montrèrent également porteurs d'un trypanosome.

Caractères d'adaptation cavernicole. Comme on l'a dit, l'*Argas boueti* se montre très caractérisé, parmi toutes les espèces d'Argasinés actuellement connues, par l'allongement de ses appendices qui dépassent légèrement la longueur du corps, au moins pour la 4ème paire, et par son rostre invaginable


1
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dans un repli tégumentaire formant gaine et susceptible de se projeter en avant, sous l'aspect d'un long suçoir exsertile.

Si l'on rapproche de ces particularités morphologiques l'allongement des appendices, l'anophtalmie (générale chez les Argas) et la faible coloration jaune *pâle* des téguments, il est permis de retrouver chez notre Argas, dans une certaine mesure, les caractères classiquement définis comme traits d'adaptation à la vie cavernicole chez les Arthropodes. Ce sont notamment les mêmes caractères que L. Fage[1] met en évidence comme correspondant à l'évolution souterraine à des degrés divers des araignées troglobies. Les particularités morphologiques saillantes de l'*Argas boueti* apparaissent ainsi comme nettement en rapport avec la vie obscuricole que mène ce parasite dans les refuges habituels des chauve-souris.

A l'appui de cette conception, il convient de faire ressortir le curieux parallélisme existant entre les modifications adaptatives présentées par l'*A. boueti* et celles d'un autre Ixodidé à biologie tout à fait analogue, l'*Ixodes vespertilionis* Koch, qui vit dans les caves et les grottes, également aux dépens des Cheiroptères. *Ixodes vespertilionis* se montre de même caractérisé, entre tous les Ixodes, par l'allongement anormal et la gracilité de ses appendices. L'armature de son rostre présente également des chélicères à digitations étirées comparables à celles d'*A. boueti*. Toutefois, chez cet Ixode le rostre n'a pas subi de transformations aussi marquées que chez notre Argas; c'est le corps entier qui a participé à l'allongement caractéristique du type cavernicole, plus prononcé chez l'*Ixodes vespertilionis* que chez l'*Argas boueti*.

A ce détail près, ces deux types d'Acariens, dont les conditions de vie parasitaire sont extrêmement voisines, doivent être considérés comme présentant des caractères de convergence adaptative qu'il est intéressant de mettre en évidence.

EXPLICATION DE LA PLANCHE XXVIII.

Figs. 1–7. *Argas boueti*.

Fig. 1. Nymphe âgée, vue dorsalement, 1/2 gorgée. × 10.
Fig. 2. Individu en état d'inanition complet. × 10.
Fig. 3. Adulte, sub-grandeur naturelle.
Fig. 4. Femelle, vue ventralement. *O*, orifices de l'organe énigmatique.
Fig. 5. Mâle, vu ventralement.
Fig. 6. Rostre en partie dévaginé engagé dans sa gaine à la base. × 30, environ.
Fig. 7. Larve gorgée. × 20.

Fig. 8. *Argas vespertilionis*, adulte. Individu d'élevage originaire de Normandie (J. Colas-Belcour). ×

[1] *Arch. Zool. Expér.* t. LXXI, avril 1931.

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A PATHOLOGICAL CONDITION OBSERVED IN TICKS (IXODIDAE).

By N. O. OLENEV and V. S. ROZHDESTVENSKAJA.

From the Zoological Institute of the Academy of Sciences, Leningrad.

(With Plate XXIX, containing Figs. 1 and 2, and Figs. I–III in the Text.)

When studying a collection of ticks sent to us by Dr G. I. Sorokoumov from Turkestan, we observed several specimens which presented a remarkable appearance. Their bodies were covered with numerous oval, whitish, well-defined elevations with a glossy surface. We could find no reference to such a condition in the literature on ticks. A disease in *Rhipicephalus bursa* nymphs, observed by P. Schulze in 1925, and supposed to be due to a fungus, offers no similarity.

The ticks with which this paper deals were of two species: *Hyalomma* sp. 3 ♀♀, and a *Dermacentor niveus* ♀ (the ♀♀ of both species were slightly gorged) found attached to horses at Dubun, Dzharkent District (South-East Kazakstan) on 22. v. 1932.

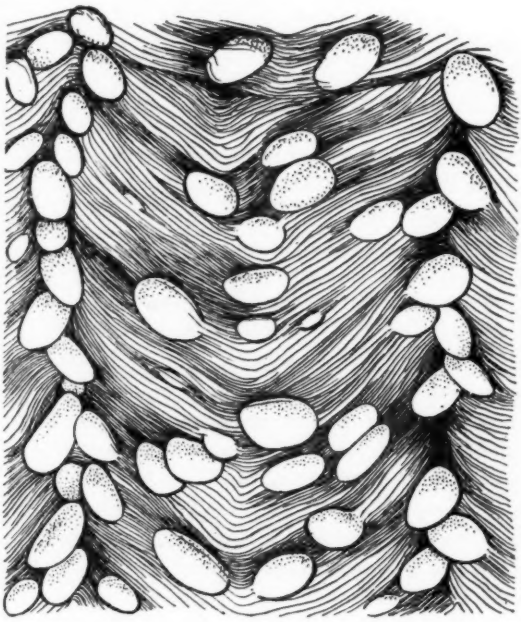
Of the three affected *Hyalomma* ♀♀, two showed many milky white elevations, hereinafter termed papules (Pl. Fig. 1), whilst one had a few situated on the anterior part of the body. The papules occur only upon the thinly chitinised parts of the body; i.e. they are absent from the capitulum, scutum and legs. The papules vary in size (Fig. I) from small ones only visible under a dissecting microscope to large ones visible to the naked eye. In the two ticks showing many papules, these were equally numerous upon the dorsal and ventral surfaces, being also present and prominent in the intercoxal spaces and around the coxae. One tick had about 1000 papules on her body. Some papules about the coxae and on other parts of the venter were oblong (Fig. II). Many papules, especially the oblong ones, were finely striated basally as is the ticks' cuticula. On Pl. Fig. 1, the papules are shown to occur fairly regularly in two rows along the lateral grooves and in single lines in the postero-lateral grooves on the dorsum.

All three females had lost the distal 2–3 articles of either two or three legs, the stumps appearing deformed and darkly chitinised as is not infrequently observed in mutilated ticks. The tick shown in Pl. Fig. 1 shows three mutilated legs on one side. The presence of mutilated legs in these ticks may or may not bear on the origin of the papules.

Fig. 1.

Fig. 2.

The *Dermacentor niveus* ♀ (Pl. Fig. 2) had normal legs and bore about 500 papules on her body, but they were larger and more rounded than those in *Hyalomma* and not definitely milkish white. Fig. III illustrates a section through a papule excised from the tick's venter; it shows that the papule consists of chitin.



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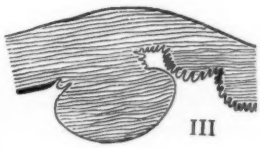

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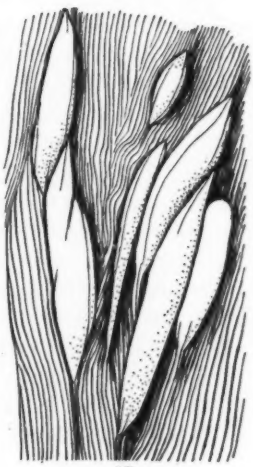

II

Hyalomma sp., ♀.

Fig. I. Papules on dorsum.
Fig. II. Papules on venter near capitulum.
Fig. III. Papule from ventral integument as seen in section.

Many ticks derived from different regions of our country have been examined by us during the past ten years, but we have not hitherto observed a like appearance. Its cause and significance remain to be explained.

EXPLANATION OF PLATE XXIX.

Fig. 1. *Hyalomma* sp., ♀, in dorsal aspect, showing papules. Three legs on one side deformed through mutilation.

Fig. 2. *Dermacentor niveus*, ♀, in dorsal aspect, showing fewer papules, these being less defined, but larger and more rounded.

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ACESPADIA POMPOSA N.G. ET SP. A PARASITIC COPEPOD OF *RHYNCODON TYPICUS*.

By W. HAROLD LEIGH-SHARPE, M.Sc. (Lond.).

(With 2 Figures in the Text.)

Habitat and material. By the courtesy and at the request of Prof. Vladimir Ebathof of Archangel I have been enabled to examine and describe two female copepods belonging to a new genus and species of the family Lernaeopodidae, consigned to him as taken from the gills of *Rhyncodon*[1] *typicus* Smith caught in 1913 in the Pacific between China and south-west Japan. One specimen, drawn to scale (Fig. 1), was twice the size of the other.

Body. The outline of the animal is best seen from Fig. 1. The cephalothorax is tilted slightly backwards, strongly flattened dorsi ventrally and bears a well-marked carapace. *Neck* somewhat elongated, slender anteriorly, much enlarged at the base where the second maxillae fuse with it, and bearing a ventral projection. Length two-thirds that of the trunk. No signs of segmentation. *Trunk* clavate, broader anteriorly, very narrow posteriorly with a dorsal projection. Genital segment well marked, resembling a much-flattened sphere. From this emerge the ovisacs which are large, clavate and broader posteriorly, nearly as long as the trunk and contain numerous small eggs. There is a small unsegmented abdomen which bears no appendages. Mouth cone prominent. Colour, yellowish white in the alcohol-preserved specimens.

The **appendages**, all paired, are (Fig. 2):

Antennules (Fig. 2 *A*1.), four-articled, basal article enlarged, distal article the largest capped by one large and three small spines. The article next the basal bears a prominent seta.

Antennae (Fig. 2 *A*2.) consist of a basal article bearing a large bluntly-ending endopodite, and a small tapering exopodite devoid of setae.

Mandibles (Fig. 2 *Mn.*) long and slender: dental formula H5, Z1, H1, N3.

1*st maxillae* (Fig. 2 *Mx*1.) with tripartite endopodite and a well-developed exopodite, two-articled, the distal article with three spines.

2*nd maxillae* (Fig. 1 *Mx*2.) long, tapering, much longer than the trunk, unwrinkled, attached to the cephalothorax at the base of the neck far below the bases of the maxillipedes in such a way as to form a collar. This position of the second maxillae would make this genus fall in Wilson's subfamily Clavellinae, but the other appendages are almost exactly like those of *Lernaeopoda* and these maxillae are longer than the cephalothorax. The arms of the maxillae grade into the pale, slightly-chitinised bulla which is spatulate, hence the generic name, so that it is difficult to tell at what point the substance of the

[1] Jordan considers this a misprint for *Rhineodon*: most authors write it *Rhinodon*.

Fig. 2.

Fig. 1. *Acespadia pomposa* n.g. et sp. ♀ in right lateral aspect. *C.* = cephalothorax; *N.* = neck; *Mx.2.* = second maxilla; *B.* = bulla; *Tr.* = trunk; *G.S.* = genital segment; *A.* = abdomen; *Os.* = ovisacs.

Fig. 2. *Acespadia pomposa* ♀, the appendages (highly magnified). *A1.* = antennule; *A2.* = antenna; *M.* = mouth; *Mn.* = mandible; *Mx1.* = first maxilla; *Mp.* = maxillipede.

Fig. 1.

bulla commences. The bulla is asymmetrical as to the attachment of the maxillae (Fig. 1 *B.*). Its position resembles that of an elongated object held between a person's hands, not opposite to one another, the left hand turned downwards the right hand turned upwards. The bulla is channelled as in *Clavella*, four channellings corresponding to each maxilla.

The *Maxillipedes* (Fig. 2 *Mp.*) consist of a large, stout basal article with two cushions of spines, but with no large spine or protuberance between them, and a terminal article ending in a large curved claw with two accessory claws at its base. This article on its inner side bears a short process ending in a blunt spine as in *Ommatokoita*.

Generic characters of female. Cephalothorax tilted backwards and with a distinct dorsal carapace. Appendages resembling those of *Lernaeopoda* except the second maxillae which arise from a collar around the base of a somewhat long neck, as in the Clavellinae, are separate and connected to a channelled bulla. Neck without traces of segmentation. Genital segment well marked. Egg-strings long, eggs small and numerous. Abdomen small, unsegmented, without appendages. The terminal article of the maxillipede bears a lateral protuberance as in *Ommatokoita*.

Host. *Rhyncodon typicus*.

Males unknown.

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A CYSTICERCOID FROM *HELODRILUS* (*ALLOLOBOPHORA LONGUS* CEDE) AND *LUMBRICUS TERRESTRIS* L.

By W. F. HARPER, Ph.D., M.B.

From the Department of Anatomy, University College (University of St Andrews), Dundee.

(With 1 Figure in the Text.)

The following is a description of a Cysticercoid obtained during investigation of the larval cestode fauna of Dundee and Arbroath districts (Harper, 1930). The Cysticercoid was first obtained from *Helodrilus* (*Allolobophora longus* Cede)

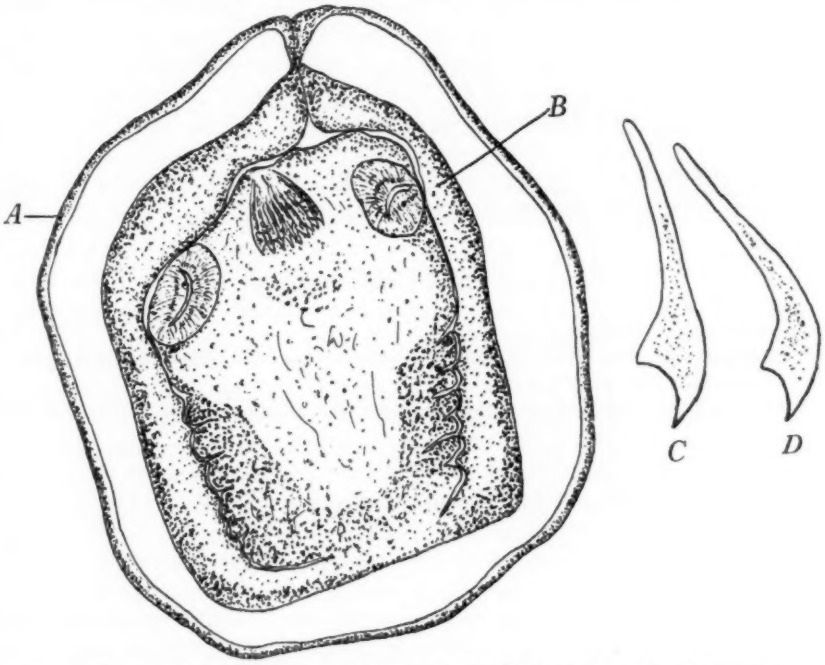

Fig. 1. Cysticercoid from *Helodrilus* (*Allolobophora longus* Cede). Sketch to show integuments (*A*, outer; *B*, inner) and two suckers. *C*, hook from distal row. *D*, from proximal row.

the host containing four larvae; later, two similar larvae were found in *Lumbricus terrestris* L. Many specimens of these hosts were dissected but only the above two infections were observed.

The parasites occurred in the coelom about its middle, and there was a slight degree of fusion to the body wall of the host. The Cysticercoid is roughly spherical in outline with a diameter of 1–1·25 mm. The colour is greyish white and little of the internal structure can be made out in the living state. Serial sections show a backwardly drawn scolex surrounded by two integuments. The outer integument (Fig. 1 *A*) is thin, but tough, and is separated from the inner (Fig. 1 *B*) except at the anterior extremity opposite the scolex. The latter is armed with four relatively small suckers (approx. diameter 0·16 mm.) and fifty hooks arranged in a double row. The average measurement of the hooks is 0·09 and 0·098 mm., in the proximal and distal row respectively.

Identification.

In the absence of direct animal experiment by feeding the Cysticercoid to a suitable adult and in this way securing the corresponding adult, it is not possible to assign it definitely to any known Cestode. All that can be stated in the present case is that the number, shape and measurements of its hooks agree closely with those of *Dilepis undulata* Schr., a common parasite of blackbirds, thrushes, starlings and other birds. It might be noted further, that even the identification of certain adult Cestodes including the above species has frequently presented difficulties (*vide* Neslobinsky (1911) and Rosseter (1906)).

My thanks are due to Prof. D. Rutherford Dow of this Department, and to Dr J. Stephenson who named the oligochaete hosts.

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(*MS. received for publication* 19. v. 1933.—Ed.)

ON TWO NEW CESTODES FROM THE AMAZON SILUROID FISH *BRACHYPLATYSTOMA VAILLANTI* CUV. AND VAL.

By W. N. F. WOODLAND.

Wellcome Research Institution, Euston Road, London, N.W. 1.

(With Plate XXX, containing Figs. 1–22.)

Brachyplatystoma vaillanti is locally very common in the Amazon river, though, during my collecting tour in 1931, I examined only twenty-two examples, caught between Codajaz (nearly 1200 miles from the sea) and Gurupa. The local name of this Siluroid is Piramutāb; it is a fairly large fish (my largest specimen measuring 67 cm. in length) and possesses very long maxillary barbels (in some cases nearly as long as the body). From this fish I obtained two new species of Cestodes, one, the more numerous parasite, is a Phyllobothriid, and, so far as I am aware, this is the first occasion on which a Phyllobothriid has been described both from a fresh-water fish and from a Siluroid; and the other is a second and new species of Fuhrmann's genus *Goezeella*, very closely related to his *G. siluri* described in 1915.

Anthobothrium piramutāb sp.n.

(Figs. 1–8).

Numerous examples of this species were present in four out of the twenty-two fish examined, the four infected fish being caught between Codajaz and Parintins. My largest worm measured about 60 mm. in length, with a maximum breadth (more or less posteriorly) of 1·23 mm. The strobila is fairly uniform in breadth, the maximum breadth only being reached very gradually, and the segments are very numerous; the posterior gravid proglottids are very elongated and relatively narrow. The strobila behind the small scolex may form a narrow "neck" or may undergo no diminution of breadth, and the proglottids behind the scolex very quickly become mature, so that proglottids with well-developed uteri are found within 10 mm. of the scolex. Mature proglottids with well-developed uteri containing lateral outgrowths full of eggs which have not opened to the exterior (Fig. 4) are approximately square in outline (about 1 mm.), but more posteriorly, when the uterus pore has been formed and the eggs discharged, the proglottids attain a length of 3 mm. corresponding to a breadth of 0·8 mm. (Fig. 6). The cirro-vaginal apertures are irregularly alternate in arrangement and the uterus pores extend over the greater part

of the length of the segment—from in front of the ovary to near the anterior limit of the proglottid.

The scolex, like the strobila, is contractile and its shape and dimensions vary according to the state of contraction (Fig. 3). It consists of a central pillar bearing four typical phyllidia which take the form of thick-walled cups (the cavities are the bothridia) of very definite form (Figs. 1, 2), and a small rostellum which however does not contain a sucker, though a group of gland (or sense) cells appears to be present. True suckers are entirely absent. On an average the scolex (from the base of the phyllidia to the apex of the rostellum) measures about 0·25 mm. in height and 0·28 mm. broad (between bases of phyllidia), but these dimensions may vary considerably, as already stated.

A typical mature proglottid with a well-developed uterus as yet devoid of an opening to the exterior has the following structure (Figs. 4, 5). The testes are roughly about 80 in number and in surface view measure about 40 microns in diameter (up to 50 microns in transverse sections). They are disposed on both sides of the proglottid (internal to the vitellaria and anterior to the ovary and leaving a wide unoccupied space in the middle line), though there is a break in continuity in the vicinity of the cirrus and vagina. Like all other organs, the testes are medullary in position, *i.e.* lie internal to the zone of longitudinal muscle fibres which, in this species as in most or all Phyllobothriids, do not form a definite band separating medullary from cortical parenchyma but the individual fibres of which are scattered on the inner side of and actually in the subcuticular layer—a zone of clear cortical parenchyma thus being absent. The cirrus sac is of the common piriform shape, is about 0·21 mm. long and 0·09 mm. broad and extends inwards (between the dorsal and ventral excretory canals) to about one-fifth the proglottid breadth. The wall is apparently muscular and the cirrus is also thick-walled and unarmed. In none of my preparations is the cirrus extruded. The vas deferens forms a mass of convolutions immediately after leaving the sac. The vagina opens (usually) anteriorly to the cirrus sac and at the same horizontal level, the two opening into a shallow atrium. The vagina is dilated at its opening and has gland cells on its wall but soon becomes and remains a comparatively narrow tube which, as usual, runs down the middle line, dorsal to the uterus, to the isthmus of the ovary. The ovary consists of two lateral masses of follicles united by a median "isthmus." This tapeworm being an undoubted Phyllobothriid, I had expected the ovary, in my transverse sections, to show a distinct quadripartite or X-shaped form, but, unless a partly bilaminate condition of the ovary mass represents a trace of this condition, there is no evidence that this is the case, and I can only suppose that the depth of the proglottid affords insufficient space. The uterus originates, as usual, as a forward tubular growth in the median line and it soon develops numerous lateral diverticula which extend about half-way to the proglottid margin. The uterus becomes filled with eggs as soon as the pockets appear, but it is not until the proglottid has become posteriorly situated and elongated in form that the ventral uterus wall, along

the greater part of its length, coalesces with the ventral subcuticula and opens to the exterior, and immediately discharges the majority of the eggs (Figs. 6, 7). This shallow elongated type of uterus pore is similar to that which Beddard described in his "*Solenotaenia*" *viperis*—a typical Proteocephalid—and it is also found in *Anthobothrium cornucopia* and *Dinobothrium septaria* (Phyllobothriids). The vitellaria (about half the diameter of the testes in surface view) are of the typical Phyllobothriid kind, *i.e.* vitellaria marginal in position and arranged more or less in the form of two semicircles in transverse sections. The two main vitelline ducts connecting the vitellaria with the isthmus genital complex pass across the proglottid posterior to the uterus pore and at the level of the isthmus. I have not ascertained the details of the genital complex. The excretory system consists in part of the usual two pairs of lateral canals—small thick-walled dorsal and large thin-walled ventral—which become so small anteriorly that I cannot trace them into the scolex. I can find no transverse connecting canals, but in each segment, in the ovary region, both ventral canals communicate with the exterior by several distinct though minute foramina secundaria opening on the ventral surface. The longitudinal muscle system consists of a layer (more than one deep in places) of very fine muscle fibres lying immediately underneath the cuticle—the cuticular longitudinal muscle layer—and irregularly placed much larger fibres situated mostly at the base of the subcuticula at its line of junction with the parenchyma but also to some extent actually in the subcuticula. These fibres, in this species, are not very numerous and there is no attempt at bundle formation. It is evident then, that as in all or most Phyllobothriids, there is no clear cortical parenchyma distinct and separate from the subcuticular layer. I have observed no calcareous corpuscles in the parenchyma. Finally, the eggs contained in the uterus (and examined in the formalin-glycerine mixture in which they were fixed and preserved) are approximately spherical and are thin-shelled, with no special "poles" to the shell (Fig. 8). The outer shell measures 22–26 microns in diameter and the hookless embryo 11–12 microns.

That this tapeworm is a Phyllobothriid, though the first to be described from a fresh-water fish and a siluroid, is proved by the phyllidia-bearing scolex, the absence of a clear cortical area of parenchyma, the longitudinal muscle fibres being scattered and lying next and in the subcuticula and not forming a definite layer, and by the marginal situation of the vitellaria, these forming two semicircles in transverse sections (*vide* Woodland, 1927). Two features common to many Phyllobothriids, viz. the quadripartite or X-shaped ovary (in transverse sections) and a uterine duct (leading from the ovary to the sac), are not present in this species. Assuming that this worm is a Phyllobothriid, the only genus listed by Southwell (1925) which will receive it is *Anthobothrium*, the diagnostic features of which are that the scolex is unarmed, the four phyllidia possess entire or crenulate margins, the bothridia are not subdivided into areolae and accessory suckers are absent. It is true that the scolex of the present species is widely different from those of other species of *Anthobothrium*

and of other unarmed Phyllobothriids, and more closely resembles some of the scoleces of the armed forms ("Onchobothriidae"), but the differences afford no basis for founding a new genus.

Goezeella piramutāb sp.n. (Figs. 19–22) and **G. siluri** Fuhrmann (1915) (Figs. 9–18).

Before referring to the new species of *Goezeella* which I found in *Brachyplatystoma vaillanti*, I will provide some notes on the species first described by Fuhrmann—*Goezeella siluri*. Fuhrmann based his description of *G. siluri* on four specimens which he found in a formalin-preserved example of the small siluroid Fish, *Cetopsis caecutiens*, from the Amazon. I also examined one example (fresh) of this Fish, the local name of which was given to me as "Kāndeeru" and which was caught at Gurupa. This Fish, which was adult though it only measured 23 cm. in length, was apparently *C. caecutiens* and not the allied species *C. candiru*, since the dorsal fin terminated in a flexible bristle (a distinction not shown in the illustrations in Spix and Agassiz). Fuhrmann's four specimens of *Goezeella siluri* were all about 8 cm. long and apparently all of much the same appearance: in all the proglottids were apparently fairly thick (dorso-ventrally) and varied in length between 0·47 and 1·1 mm. and in breadth between 1·6 and 2 mm. In my example of *Cetopsis caecutiens* I found twenty-three worms which, however, were all much smaller than those described by Fuhrmann, the largest not exceeding 4·5 cm. in length with a maximum breadth of 1·8 mm., and in the form of strobila they differed to such an extent as to lead me at first to believe that I was dealing with two distinct species. In six specimens (the longest about 3 cm.) the strobila was of the solid type described by Fuhrmann and transverse sections resembled those which he figured, but in the majority of the remaining specimens the proglottids were very much thinner (due to the attenuation or almost entire absence of the longitudinal muscle layer) and anteriorly were expanded laterally so as to give this region of the strobila the appearance of the "hood" of a cobra (Fig. 12). These thin proglottids were usually more expanded than the thick ones but were not expanded nearly to such an extent as would be required to attenuate the muscle layer almost to a state of invisibility (compare Figs. 13 and 16 with Figs. 14, 15, 17). Thus in portions of a young thick worm the proglottids on the average measure 1·29 mm. in breadth and 0·47 mm. in length, but in a "cobra" form the proglottids of the "hood" measure 2·04 mm. in breadth and only 0·11 mm. in length; immediately behind the "hood" the proglottids narrow to 1·32 mm. broad and 0·49 mm. long, and further back proglottids of the same breadth elongate to 1·12 mm. The evidence that the thin "cobra" forms are of the same species as the thick forms is to be found in the similar scoleces, the similar arrangement of the organs in the proglottids, the existence of transitional forms between the thick and the "cobra" types (Figs. 10, 11), and the fact that the eggs of both types (examined in the undistorted condition) are identical in shape and size.

Fuhrmann does not provide a figure of the intra-uterine eggs and only remarks that they are of an oval form, the embryos measuring 0·01 × 0·012 mm. and the external shell 0·023 × 0·025 mm., and that the shells are devoid of the polar cone or knob-like projections seen in the eggs of *Corallobothrium lobosum*. The form of the undistorted eggs is shown in Fig. 18. These figures were drawn while the eggs were still in the formalin-glycerine mixture which I used for fixing and preserving the worms in Brazil. I have nearly always found that transfer of the worms to any other liquid at all different in composition leads to a crumpling of the egg-shell and its contents, and my first act in examining a worm is to draw the eggs from a gravid proglottid. I have confirmed Fuhrmann's measurements for the eggs (of both the thick and "cobra" types of worm). As Fuhrmann remarks, the poles of the shells do not bear knobs (or conical projections) but, on the other hand, each has a most distinct circular opening, through which there extends a long (about three-quarters of the length of the shell) delicate hyaline filament. This filament was not observed by Fuhrmann but is quite constant in all the eggs I have examined.

As already mentioned, I found (hind intestine) in one specimen of *Brachyplatystoma vaillanti* (caught about 70 miles below Manaos) one entire specimen and two strobilae of a worm which answers well to the bulk of the description given by Fuhrmann for *Goezeella siluri* and yet, judging by the shape and size of its intra-uterine eggs and perhaps also certain minor indefinable differences, is a distinct species. The entire worm measured about 55 mm. in length, with a maximum breadth of 1·5 mm. The scolex (Figs. 19, 20) measured 1·35 mm. in breadth (in region of "collar") and 0·88 mm. in length (from the base of the "collar" to the apex) and is very little broader than the adjoining strobila, which therefore shows no signs of a narrow "neck." The scolex is unarmed and, above the "collar," pyramidal in form with a bluntly pointed apex and carries four ordinary suckers (*ca.* 0·36 mm. deep and *ca.* 0·39 mm. in transverse diameter). The base of the scolex takes the form of a feebly developed (in this particular specimen) collar, the surface of which is only slightly wrinkled. Since, as I have remarked, the anatomy of this worm (Fig. 21) is substantially the same as that of *G. siluri*, it will suffice if I provide a figure (Fig. 22) and measurements of the only character which easily and clearly differentiates it, viz. the intra-uterine egg. These eggs, like those of my specimens of *G. siluri*, were examined while still in the formalin-glycerine mixture employed to fix and preserve the worm in Brazil and were quite undistorted. If Figs. 22 and 18 be compared (drawn to the same scale), it will be seen that the eggs of the Piramutāb species are much smaller than those of *G. siluri* (the external shell measuring about 18 × 13 microns and the ovoid embryo about 16 × 10 microns, whereas the corresponding measurements of the eggs of *G. siluri* are, in both the thick and "cobra" forms, about 25·6 × 20 microns and 11 × 11 microns) and the external shell is more simple in form (no obvious terminal openings being visible and projecting filaments being absent). I suggest that this new species of *Goezeella* be named *G. piramutāb*. A complete diagnosis must await the examination of further material.

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EXPLANATION OF PLATE XXX.

Anthobothrium piramutāb sp.n. (Figs. 1–8).

Fig. 1 (×56): the scolex. Fig. 2 (×56): a phyllidium showing the shape of the cavity (bothridium). Fig. 3: diagram showing different forms of the scolex. Fig. 4 (×39): a young gravid proglottid. Fig. 5 (×56): transverse section of young gravid proglottid through hind end of ovary. Fig. 6 (×18): posterior gravid proglottid which has shed its eggs through the elongated uterus pore. Fig. 7 (×56): transverse section of posterior gravid proglottid to show the uterus pore and degeneration of other organs. Fig. 8 (×395): eggs drawn in the original formalin-glycerine preserving fluid.

Goezeella siluri (Figs. 9–18).

Fig. 9 (×12): a "normal" scolex. Figs. 10, 11 (×12): scoleces of worms more or less intermediate in form between the "normal" (described by Fuhrmann) and the "cobra" type. Fig. 12 (×12): the "cobra" type of worm with expanded segments almost entirely devoid of the longitudinal muscle layer. Fig. 13 (×12): gravid proglottids of the "normal" thick type of worm. Figs. 14, 15 (×12): gravid proglottids of the "cobra" type of worm. Fig. 16 (×28): transverse section through gravid proglottid of "normal" thick worm. Fig. 17 (×28): transverse section through gravid proglottid of "cobra" type of worm. Fig. 18 (×395): eggs from the "normal" (thick) and "cobra" types drawn in the original formalin-glycerine preserving fluid. The terminal filaments are in reality more hyaline in appearance than shown.

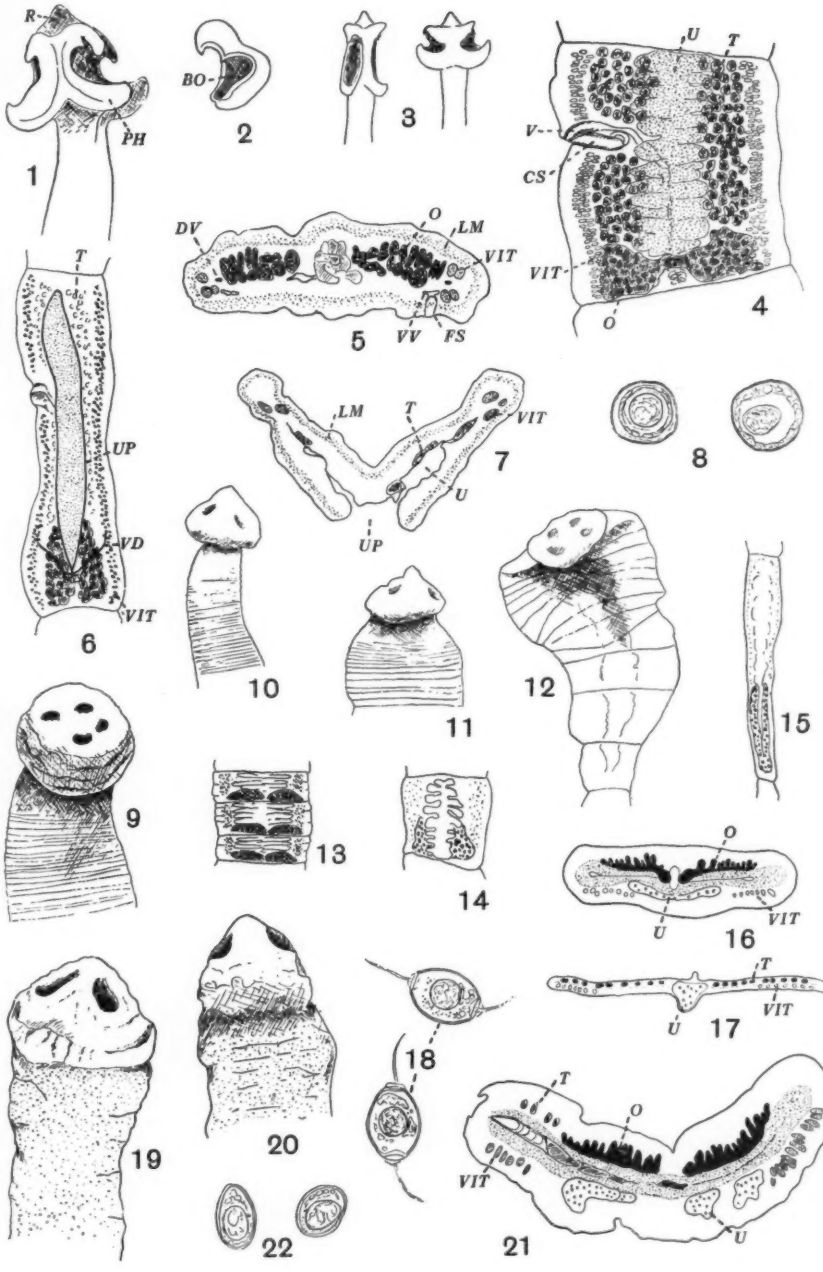
Goezeella piramutāb sp.n. (Figs. 19–22).

Figs. 19, 20 (×18): two aspects of the same scolex. Fig. 21 (×39): transverse section through gravid proglottid (substantially identical with that shown in Fig. 16). Fig. 22 (×395): the eggs, conspicuously different from those of *G. siluri*.

Lettering to Figures.

BO, bothridium; *CS*, cirrus sac; *DV*, dorsal vessel; *FS*, foramina secundaria; *LM*, longitudinal muscles; *O*, ovary; *PH*, phyllidium; *R*, rostellum; *T*, testes; *U*, uterus; *UP*, uterus pore; *V*, vagina; *VD*, vitelline duct; *VIT*, vitellaria; *VV*, ventral vessel.

(*MS. received for publication* 21. IV. 1933.—Ed.)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ON A NEW SUBFAMILY OF PROTEOCEPHALID CESTODES—THE OTHINOSCOLECINAE—FROM THE AMAZON SILUROID FISH *PLATYSTOMATICHTHYS STURIO* (KNER).

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(With Plate XXXI, containing Figs. 1–20, and Fig. I in the Text.)

Othinoscolex lenha, gen. et sp.n. (Pl. figs. 1–6).

I EXAMINED altogether five specimens of *Platystomatichthys sturio* (Kner), locally known as the "Peixe Lenha," a large Siluroid, slaty grey or dark green in hue with black spots, a flat head with a projecting spatulate upper jaw and long maxillary barbels. The largest specimen measured 110 cm. in length. A smaller specimen (only 58 cm. in length) caught fifty miles below Santarem, contained, in addition to the new species of *Monticellia* and *Proteocephalus* described below, three specimens of a Cestode which is so distinctive in structure that it must serve as the type of a new subfamily of the Proteocephalidae.

The largest of the three Cestodes is (in formalin) about 40 mm. long, and, save where the strobila tapers slightly at the fore and hind ends, 2·2 mm. broad, and the other two specimens were only slightly smaller. The segments are numerous and very flat and all are broader than long, even the longest gravid segments being only 0·54 mm. long and 1·6 mm. broad; externally the primary segmentation is masked by secondary transverse creases in the cuticle and subcuticula. There is no neck, the region of strobila following the scolex being broader than the scolex and segmented. The last five or six terminal segments are all smaller than the preceding segments, tapering almost to a point, which indicates that the segments are not detachable. In none of the segments are the uterine pores present, hence they are not completely developed, though the uteri are full of eggs. The cirro-vaginal apertures are of course marginal and irregularly alternate, and open at or very near to the anterior border of each segment (Fig. 3). The vaginal opening is in front of the cirrus sac.

The scolex (Fig. 2) to a large extent resembles that of *Peltidocotyle rugosa*, but it is entirely devoid both of the bilocular or rather biporal suckers figured by Diesing (1855) and the spatula-shaped areas described and figured by me (1933). It consists simply of an irregular mass of fairly thick processes and foldings of the subcuticula in this region, each containing a core of parenchyma. No suckers or special adhesive areas have been detected either by direct

examination or by the study of a series of sagittal sections. In external appearance and size the scolex varies to a certain extent according to the state of contraction of the processes. In the three specimens it measures 1·0–1·5 mm. in diameter and about 0·8–1·4 mm. in length.

A young gravid proglottid (Figs. 3–5) has the following structure. The testes are roughly about 100 in number and lie in one field situated between the lateral vitellaria, in front of the ovary and above the uterus, and, in transverse sections, measure about $58 \times 40 \mu$. The testes form a layer one deep and are situated in the dorsal cortex, lying outside the layer of longitudinal muscle bundles. The cirrus sac is about $332 \times 182 \mu$ (in transverse sections) and contains the thick stumpy unarmed cirrus, with a few coils of the vas deferens. The sac wall is thin and apparently muscular. The vas deferens, on emerging from the sac, forms a small mass of coils. The vagina, as already stated, lies and opens in front of the cirrus sac and is slightly dilated and thick-walled next the opening. It runs straight to the middle line and then bends at right angles, above the uterus, to end in the ovary isthmus. The cirrus sac, vas deferens and vagina lie, as usual, in the medulla. The ovary is of the normal kind and is shown in Figs. 3 and 4. It is situated in the medullary parenchyma. The uterus is also of the normal type with diverticula but is decidedly small in size, the total breadth only being a little more than one-fifth of the breadth of the proglottid. It is filled with eggs but has not formed pores or a pore to the exterior and is situated in the ventral cortex in the median line. The intra-uterine eggs are not spherical but somewhat irregular, and the embryos are always oval. The outer shell is thin and about 21μ in diameter; the embryo measures about $15 \times 8 \mu$ and is hookless. The vitellaria lie in the marginal cortex in four short rows, one dorsal and one ventral on each side (Figs. 4, 5). The vitellaria are $44 \times 33 \mu$ in transverse sections. The excretory system is not conspicuous and shows two small vessels on each side in the medulla situated internal to the vitellaria; they lie very close together and the ventral is slightly larger than the dorsal. The longitudinal muscle system is formed of a single thin layer of small bundles consisting of closely packed fibres, the bundles having the arrangement diagrammatically shown in Figs. 4, 5. Apart from the cirrus sac, vas deferens and vagina which are always medullary, *i.e.* internal to the longitudinal muscle layer, all the genital organs except the ovary are situated in the cortex. The medullary area of parenchyma is in consequence very small compared with the cortical. Calcareous corpuscles are absent.

Systematic position.

In a separate publication (Woodland, 1933) I have suggested a new basis for the classification of the Proteocephalidae, the family being subdivided into five subfamilies according to the arrangement of the principal genital organs relative to the longitudinal muscle layer. These five subfamilies can be defined as follows:

Proteocephalinae Mola 1929. Proteocephalids in which the testes, ovary, vitellaria and uterus are not situated external to an internal layer of longitudinal muscles or other line of demarcation of the medulla from the cortex[1], and the vitellaria are compactly arranged around a central duct in two marginal strands. Genera: *Proteocephalus* Weinland 1858, etc.

Zygobothriinae Woodland 1933. Proteocephalids in which the vitellaria alone are cortical in position and are dispersed and placed dorsally and ventrally towards the sides of the segment. Genera: *Zygobothrium* Diesing 1850, *Amphoteromorphus* Diesing 1850.

Marsypocephalinae Woodland 1933. Proteocephalids in which the testes alone are cortical in position and the medullary vitellaria are as in the Proteocephalinae. Genus: *Marsypocephalus* Wedl 1862.

Ephedrocephalinae Mola 1929. Proteocephalids in which both the testes and vitellaria are cortical in position, the vitellaria being dispersed and situated in two ventral rows lying towards the sides of the segment. Genus: *Ephedrocephalus* Diesing 1850.

Monticelliinae Mola 1929. Proteocephalids in which the testes, vitellaria, uterus and the whole or the greater part of the ovary are in the cortex. The vitellaria are dispersed and are marginal or ventral in position. Genera: *Monticellia* La Rue 1911, *Goezeella* Fuhrmann 1916, *Peltidocotyle* Diesing 1850.

It is evident from the preceding definitions that the worm just described presents a new type of arrangement; in fact, it fills up the gap between the Ephedrocephalinae, in which testes and vitellaria alone are cortical, and the Monticelliinae in which all these organs are cortical, by exhibiting a condition in which all the organs are cortical save the ovary (vide Fig. I). I propose to name this worm *Othinoscolex lenha*, having regard to the unusual character of the scolex and the local name of the host, and the new subfamily will therefore be known as the Othinoscolecinae.

The definition of the new subfamily Othinoscolecinae is as follows:

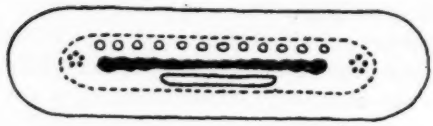
Proteocephalids in which the testes, vitellaria and uterus are cortical, only the ovary being medullary. The vitellaria are dispersed and are arranged as in the Zygobothriinae.

A provisional definition of the new genus *Othinoscolex* is as follows:

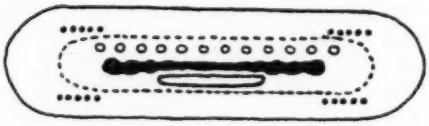
Othinoscolecinae. Scolex large, the surface bearing folds or processes of the cuticle and subcuticula with a core of parenchyma; suckers present[2] or absent; no apical organ. Neck absent. All segments broader than long. Cirro-vaginal pores irregularly alternate, and the vagina opens in front of the cirrus sac.

[1] This mode of expressing the medullary situation of all the organs is adopted in order to include forms devoid of any distinction between cortex and medulla.

[2] To include the second species, *Othinoscolex myzofer*, described below.



PROTEOCEPHALINAE



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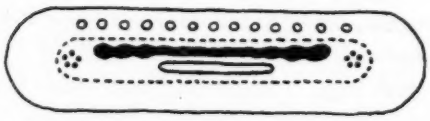

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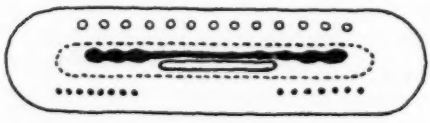
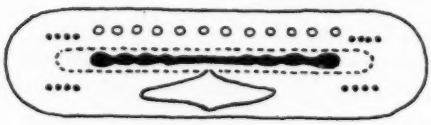
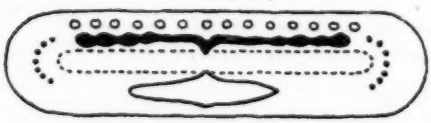


EPHEDROCEPHALINAE



OTHINOSCOLECINAE



MONTICELLIINAE

Fig. I.

Othinoscolex myzofer, sp.n. (Pl. figs. 7–9).

In two other specimens of *Platystomatichthys sturio* caught in the one case ten miles below Itacoatiara and in the other about eight miles below Obydos I found in each about a dozen specimens of a medium-sized worm with a large wrinkled scolex which represents a distinct second species of the new genus *Othinoscolex*. My largest example of this new worm was about 30 mm. long with an almost uniform breadth of 1·8 mm., the strobila only narrowing in the neck region and towards the posterior extremity. Externally the true segmentation is masked by the presence of secondary transverse grooves on the proglottid and the subcuticula is so thick that it is difficult to prepare transparent whole mounts. Horizontal sections show that all the proglottids are much broader than long, some of the most gravid in my collection being 1·1 mm. broad and only 0·29 mm. long. The scolex (Fig. 7) is large (maximum about 2·3 mm. broad and about 1·1 mm. long, when the anterior cone is not projected) and consists of a wrinkled broad base carrying a terminal small cone bearing four large suckers. There is no apical organ. The strobila may or may not be constricted behind the scolex and shows segmentation extending up to the base of the scolex, so that properly speaking there is no neck. The segments are extremely numerous and the terminal ones are small compared with those preceding and are not detachable. The cirro-vaginal apertures are marginal and irregularly alternate and open in the anterior third of the segment.

The anatomy of a gravid proglottid is as follows (Fig. 8). The testes are roughly about 150 in number and are arranged in the dorsal cortex in a single field, one layer deep, lying in front of the ovary and between the vitellaria and anterior border of the segment. The testes measure in transverse sections about $102 \times 51 \mu$. The cirrus sac ($365 \times 83 \mu$) has a thin dense muscular wall. The cirrus is unarmed and contains a terminal convoluted ductus opening into a thick-walled straight proximal portion. The vas, on leaving the sac, forms a convoluted mass. The vagina, as in *Othinoscolex lenha*, lies at its origin in front of the cirrus sac, the portion next the sac being slightly dilated, and runs transversely to the middle line where it bends back to reach the ovary. The ovary has the usual form and lies in the medulla. The uterus is small, extending laterally over less than one-third of the segment, and consists of a median stem with small lateral diverticula, lying in the ventral cortex. Uterus pores are not present in the material I have examined but doubtless an elongated pore is formed in the usual way. The eggs (Fig. 9) are somewhat irregular in form ($23 \times 18 \mu$; hookless embryo $14{\cdot}6 \times 11 \mu$). The vitellaria are arranged in the marginal cortex in precisely the same fashion as in *Othinoscolex lenha* and, in transverse sections, measure about $70 \times 33 \mu$ as a maximum. The two main channels of the excretory system, the dorsal and the ventral of each side, are very conspicuous. The longitudinal muscle system is well marked. It consists of a layer, some four or five fibres deep, of small fibres fairly tightly

packed but not aggregated into very definite bundles; in short the bundles are ill-defined. Circular muscle fibres appear to be absent. Calcareous corpuscles are not present in the parenchyma.

This tapeworm is obviously only a second species of the new genus *Othinoscolex* just described, though it is a very distinct species. The external appearance of the worm is not unlike *Othinoscolex lenha*, the real segmentation in both cases being masked by secondary transverse grooves and all segments being broader than long and of more or less uniform width; in both species there is no neck, the vaginal pores open in front of the cirrus sacs, the uteri are small, the eggs are similar and the organisation is arranged on the same plan. It is true that the scolex of the present species bears suckers while that of *O. lenha* is entirely devoid of them, but in all other respects they are of the same type. This seems to be a clear example of a case in which the scolex must be regarded as relatively unimportant and its peculiar characters only of specific value.

The main distinctions between the two known species of *Othinoscolex* are as follows:

Othinoscolex lenha. Scolex bears an irregular mass of fairly thick processes and foldings of the cuticle, subcuticula and underlying parenchyma. Suckers or other special adhesive organs entirely absent. The strobila is thin in transverse sections, the depth being usually less than one-eighth of the breadth. Testes about 100 in number and in transverse sections of gravid proglottids measure about $58 \times 40 \mu$. The longitudinal muscle system consists of a single layer of small closely packed bundles.

Othinoscolex myzofer. Scolex consists of a large folded base carrying a small pointed projection bearing four suckers. The strobila in transverse sections is in depth at least a quarter of the breadth. Testes about 150 in number and in transverse sections of gravid proglottids measure about $102 \times 51 \mu$. The longitudinal muscle system consists of a layer of fibres (4–6 deep) only partially aggregated into bundles.

Monticellia lenha, sp.n. (Pl. figs. 10–16).

The same example of *Platystomatichthys sturio* which contained *Othinoscolex lenha* harboured also numerous specimens of the new and very distinct species of the remarkable genus *Monticellia*. It may be noted that this is the second species of *Monticellia* known to be valid, *M. coryphicephala* being the type species. Of the three other species listed by La Rue (1914) as belonging to *Monticellia*, viz. *M. macrocotylea*, *M. diesingii* and *M. malopteruri*, the last is known for certain to be a species of *Proteocephalus* and the anatomy of the other two species is for the most part unknown.

The longest worm measured (in formalin) about 30 mm. in length, with a maximum breadth (posteriorly) of about 1 mm. The segments are numerous and the vast majority are broader than long, only a few mature and gravid segments being either square in outline or at most very slightly longer than

broad (2·36 mm. long and 2·06 mm. broad). All the gravid and most mature segments develop secondary transverse creases in the cuticle but these do not obscure the primary segmentation. Segments are not detachable. The cirro-vaginal apertures are irregularly alternate and are situated in the anterior quarter of the margin; uterine pores are narrow and elongated, extending from in front of the ovary to the anterior border of the proglottid. The scolex (Fig. 10) is relatively small (0·23–0·36 mm. broad and 0·24–0·29 mm. long) and simple, consisting of a central pillar bearing four circular-rimmed suckers (0·18 mm. in diameter) and projecting slightly at the end. There is no apical sucker or other organ. One peculiarity about the scolex is that the edges and cavities of the suckers are, in all the worms examined, covered with simple spines, but in some scoleces they are very small while in others they may reach a maximum size of 3·6 μ in length. Apart from the species of *Tentacularia* (Tetrarhynchidae) from the Amazon Sting-Ray, which will be shortly described in another paper, this is the first instance, which I have met with personally, of a tapeworm from the Amazon possessing spines on the scolex. An unsegmented neck region is present, very variable in length (0·33–1·49 mm.) and breadth (0·16–0·33 mm. immediately behind scolex).

The structure of a mature-gravid proglottid (Figs. 11, 12) is briefly as follows. The testes are rather more than 200 in number and are situated in two fields. The cirrus sac (Fig. 15) in transverse sections is about 0·34 mm. long and 0·13 mm. broad and has a thin though tough wall. The contained cirrus is thick-walled and the vas deferens shows several coils inside the sac; outside the sac the narrow vas forms a number of convolutions. The cirrus is unarmed and when extruded is rather thick and blunt-ended. The vagina opens to the exterior behind the cirrus sac and has a slightly convoluted course back, dorsal to the uterus, to the ovary. The ovary is dorsally situated and from the median isthmus arises a very dilated uterine duct which opens ventrally into the middle channel of the uterus (Figs. 12, 13). The uterus, it is interesting to note, belongs to a large extent to the "Solenotaenia" type, first described by Beddard, and found in certain species of both Proteocephalidae and Phyllobothriidae. In short the cavity of the uterus is extremely shallow and, in the present species, the diverticula are very diminutive and partly solid, so that the eggs are not stored in the uterus for a long period but are liberated through the elongated split to the exterior (Figs. 12, 14) almost as soon as they arrive via the uterine duct. The eggs (examined in the original fixing and preserving formalin-glycerine fluid) are approximately spherical (Fig. 16) and have a maximum diameter of 0·0219 mm. The diameter of the hookless embryo is about 0·0146 mm. The vitellaria are arranged marginally so as to form, in transverse sections of the segments, two small and not very clearly defined semicircles or crescents, one on each side. This is the arrangement also found in *Peltidocotyle rugosa*. The excretory system is apparently of the usual type though the dorsal and ventral vessels are nearly equal in size. The system of longitudinal muscle fibres has precisely the same arrangement as in *Monticellia*

coryphicephala, but the fibres themselves are very few in number and quite insufficient to form bundles. Save at the margins the fibres, as seen in transverse sections, form a thin row one fibre deep, and only at the margins are the fibres grouped into small clusters at the centres of the crescents of vitellaria. It follows then that, as in *M. coryphicephala*, all the organs, save the vagina, cirrus sac and vesicula seminalis, are cortical in position.

It was recently shown (Woodland, 1933) that the subfamily Monticelliinae of the Proteocephalidae contains three genera—*Monticellia*, *Goezeella* and *Peltidocotyle*—solely distinguished from each other by the form of the scolex and the arrangement of the vitellaria. Judging by the scolex the present species belongs to the genus *Monticellia* which possesses a simple scolex with four circular or oval suckers, but, judging by the vitellaria, the present species belongs to the genus *Peltidocotyle* which has the vitellaria arranged as marginal crescents in transverse sections of proglottids (the vitellaria of *Monticellia coryphicephala* only form the lower halves of the crescents). It is evident therefore that since in this case the scolex character is more important or at least more evident than the slightly different arrangements of the vitellaria the latter must be omitted from the definitions of these genera and the present species referred to the genus *Monticellia*.

This new species of *Monticellia* is similar in many respects to *M. coryphicephala*, the type species, but is distinguished at once by the presence of spines on the suckers, by the more numerous testes (*M. coryphicephala* only has about 100 testes) lying in two fields (*M. coryphicephala* has the testes in one field), by the vagina opening posteriorly to the cirrus sac (the reverse in *M. coryphicephala*), by the crescentic arrangement of the vitellaria, by the "Solenotaenia" type of uterus (in *M. coryphicephala* the uterus diverticula are quite large) and by the very feebly developed longitudinal musculature (though in *M. coryphicephala* also the longitudinal muscles are apparently not strongly developed).

Proteocephalus lenha, sp.n. (Pl. figs. 17–20).

In the same individual "Lenha" (*Platystomatichthys sturio*) containing *Othinoscolex lenha* and *Monticellia lenha* I also found nearly a dozen specimens of a new species of *Proteocephalus*—*P. lenha*—which in some respects so closely resembles *Monticellia lenha* that at first I regarded them as large forms of the latter species. The resemblances relate to their scoleces, general disposition of the genitalia (position of cirro-vaginal pores, for example) and superficial appearance of the uterus, and the eggs of the two species appear to be practically identical in form and size. The points of difference will be indicated in the following description. My largest worm is about 130 mm. in length (in formalin), with a maximum breadth (about midway in the length) of about 2·59 mm. *Proteocephalus lenha* is therefore a much larger species than *Monticellia lenha*, though young specimens may bear a superficial resemblance. In *Monticellia lenha* the segments are rarely longer than broad but in *Proteocephalus lenha* all the gravid segments are longer than broad (some of the largest measuring

2·36 mm. long and 1·41 mm. broad). Eggs are shed through a narrow elongated uterus pore and the terminal segment is distinctive by reason of its rounded free end, hence the segments are not naturally detachable. As in *Monticellia lenha*, the cirro-vaginal apertures are situated in the anterior quarter of the proglottid length and are irregularly alternate. The scolex (Fig. 17) is small and strongly resembles that of *Monticellia lenha*. It is 0·19–0·28 mm. broad and 0·16–0·28 mm. long, varying greatly in size owing to different states of contraction. Similarly the suckers, when uncontracted, measure about 0·16 mm. in external diameter but may contract to 0·09 mm. I can detect no apical organ. The chief distinction between the scoleces of *Proteocephalus lenha* and *Monticellia lenha* is that the suckers of the latter are covered with spines, whereas I have not been able to discover spines on the suckers of four scoleces of the former species. There is no constricted region of the strobila behind the scolex, though it is unsegmented for a short distance (0·47–0·88 mm.) and usually becomes almost at once much broader than the scolex.

The structure of a gravid proglottid (Figs. 18, 19) is as follows. The testes are over 200 in number and are separated by the uterus into two fields. The cirrus sac is about 0·44 mm. long and 0·09 mm. broad and has a distinct muscular wall. The contained cirrus is unarmed and thick-walled and is considerably convoluted before it joins the vas deferens, which also forms a few coils in the sac; outside the sac the vas forms relatively only a small mass of coils. The vagina opens to the exterior behind the sac and is very slightly distended next the opening; its course posteriorly, dorsal to the uterus, is practically straight. The ovary is of the form shown in Fig. 18 and is hardly more dorsal than ventral in position in the medulla. The uterus consists of a large main channel, the sides of which are only slightly sacculated, and it is therefore of different type compared with that of *Monticellia lenha*. The uterine pore is formed by coalescence of the ventral wall of the uterus with the median ventral subcuticula and extends over most of the proglottid length anterior to the ovary. The eggs (examined in the original fixing and preserving formalin-glycerine fluid) are very similar, both in shape and size, to the eggs of *Monticellia lenha* (cf. Figs. 16 and 20). They are spherical in form, devoid of "poles," and about 0·023 mm. in external diameter; the hookless embryos are about 0·014 mm. The vitellaria are arranged in the normal Proteocephaline manner, *i.e.* surrounding a central duct along each margin. Two ventral excretory canals are all that I have been able to observe of the excretory system. The parenchyma is divided in the ordinary Proteocephaline manner into cortex and medulla by the presence of a thin layer of circular muscle fibres and small irregular bundles of longitudinal muscle fibres situated at intervals along the line of junction of the cortex and medulla: the arrangement therefore is quite distinct from that seen in *Monticellia lenha* and the two species must of course be placed in separate subfamilies—the Proteocephalinae and Monticelliinae respectively (*vide* Woodland, 1933).

SUMMARY.

From three specimens of the Amazon Siluroid fish *Platystomatichthys sturio* (the "Lenha") I have described (1) *Othinoscolex lenha*, representing not only a new genus and species but also a new subfamily (the Othinoscolecinae) of the Proteocephalidae, (2) *Othinoscolex myzofer*, a second species of the new genus, (3) a distinct new species of La Rue's genus *Monticellia*—*Monticellia lenha*, and (4) a new species of *Proteocephalus*, *Proteocephalus lenha*.

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EXPLANATION OF PLATE XXXI.

Othinoscolex lenha, gen. et sp.n. (Figs. 1–6).

Fig. 1 (nat. size): the entire worm.
Fig. 2 (×17): the scolex.
Fig. 3 (×27): dorsal aspect of a gravid proglottid.
Fig. 4 (×27): transverse section of a gravid proglottid in region of ovary.
Fig. 5 (×27): transverse section of a gravid proglottid anterior to ovary. The longitudinal muscles in Figs. 4 and 5 are represented diagrammatically.
Fig. 6 (×395): an undistorted egg.

Othinoscolex myzofer, sp.n. (Figs. 7–9).

Fig. 7 (×17): the scolex.
Fig. 8 (×39): transverse section of gravid proglottid in region of ovary.
Fig. 9 (×395): undistorted eggs.

Monticellia lenha, sp.n. (Figs. 10–16).

Fig. 10 (×56): the scolex, with spines on suckers.
Fig. 11 (×27): ventral aspect of mature proglottid.
Fig. 12 (×27): ventral aspect of gravid proglottid.
Fig. 13 (×39): transverse section of gravid proglottid in region of ovary.
Fig. 14 (×39): transverse section of gravid proglottid in front of ovary.
Fig. 15 (×56): the cirrus sac, with retracted cirrus.
Fig. 16 (×395): undistorted eggs.

Proteocephalus lenha, sp.n. (Figs. 17–20).

Fig. 17 (×56): the scolex.
Fig. 18 (×17): dorsal aspect of gravid proglottid.
Fig. 19 (×39): transverse section of gravid proglottid in front of ovary.
Fig. 20 (×395): the undistorted eggs.

Lettering to figures: *C*, cirrus; *CS*, cirrus sac; *DV*, dorsal vessel; *LM*, longitudinal muscles; *O*, ovary; *S*, sucker; *SP*, spines; *T*, testes; *U*, uterus; *UD*, uterine diverticula; *UP*, uterus pore; *UT*, uterus; *UTD*, uterine duct; *V*, vagina; *VIT*, vitellaria; *VV*, ventral vessel.

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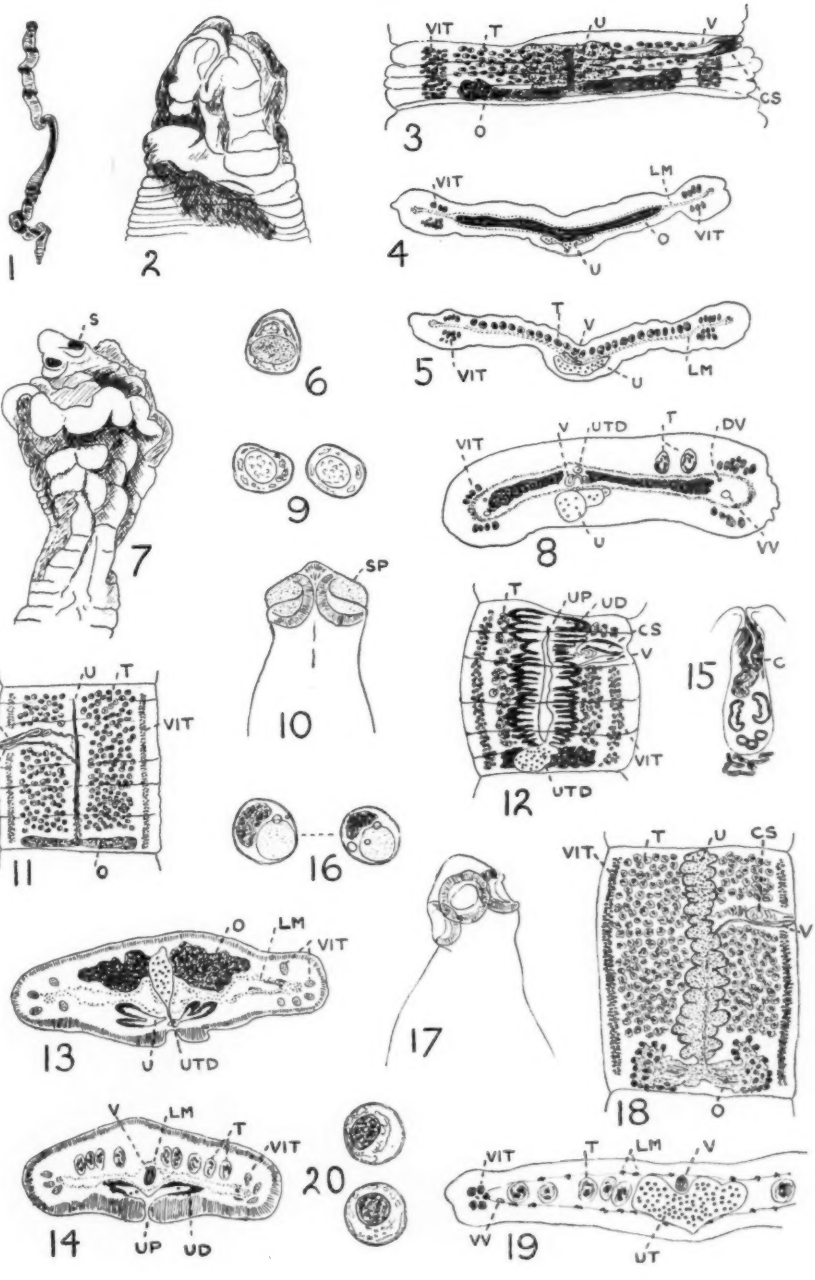
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A REDESCRIPTION OF *DISTOMUM INCOMMODUM* LEIDY, FROM *ALLIGATOR MISSISSIPPIENSIS*, AND CREATION OF A NEW GENUS (*HOMOSCAPHIS*) FOR IT.

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(With 2 Figures in the Text.)

ABSTRACT. *Distomum incommodum* (Leidy 1856) from mouth of *Alligator mississippiensis* is found to belong to the family Clinostomidae. A new genus *Homoscaphis* Canavan 1933 is erected for it, a diagnosis of which is given, with a redescription and illustration of the species.

In 1856 Dr Joseph Leidy described a fluke from the mouth of an alligator in the *Proceedings of the Philadelphia Academy of Natural Sciences*, **8**, 42–58 (reprinted on p. 85 in J. Leidy 2nd, 1904) as follows:

? *Monostomum incommodum* Leidy. Body compressed, above convex, below concave, sides parallel, anteriorly convex, posteriorly angularly convex. Head continuous with the body, obliquely truncated. Mouth round, surrounded with a wide circular lip, which is emarginate below. Male generative aperture ? communicating with a hemispherical cavity (acetabulum ?) one-fourth the length of the body from the head. Length 9 lines, breadth 1¼ lines.

Five specimens were obtained from the fauces of the *Alligator mississippiensis*, in Florida, by Prof. J. W. Bailey, of West Point. Is it probably a species of Distomum, with the ventral acetabulum emersed within the body?

In 1884, Leidy described *Distoma oricola* from the mouth of another alligator. It also was reported in the same *Proceedings*, **36**, 47–48[1], as follows:

Some of these were supposed to be leeches from the mouth of the alligator.... The present specimens, however, do not belong to a leech, but pertain to a species of *Distoma*, apparently not previously described. It may be named and be distinguished by the characters as follows:

Distoma oricola: Body elongated elliptical, moderately wider and thicker posteriorly, and ending in a blunt angular extremity, convex dorsally and flat ventrally, unarmed, smooth or minutely wrinkled transversely. Mouth subterminal, and enclosed with a reniform lip succeeded by a linear annulus. Acetabulum large, globular, included at the anterior fourth of the body, and opening ventrally by a conspicuous central aperture. Generative orifice ventral at the posterior fourth of the body. Length 15 to 20 mm.; breadth 3 mm.

Eight specimens obtained from the mouth of the alligator, *A. mississippiensis*, in Florida, by Mr Stuart Wood.

Accompanying the specimens is a fragment of the tongue marked with circular scars, apparently due to the worms. The alcoholic specimens in their present condition are incurved, with the lateral margins inverted, and the included acetabulum produces a conspicuous dorsal eminence.

[1] P. 180–181, J. Leidy 2nd, 1904.

In 1890, Leidy concluded that *Distoma oricola* Leidy 1884 and ? *Monostomum incommodum* Leidy 1856 were distomes of one and the same species. Hence we find in the November *Proceedings*, **42**, 410–418[1] the following:

"*Distomum incommodum*
? *Monostomum incommodum*, Leidy, *Pr. A.N.S.* 1856, 43.
Distoma oricola, Leidy, *ibid.* 1884, 47."

The question mark (?) above is Leidy's as it appears in the original description. But an erroneous date 1858 for 1856 appears in the compilation of J. Leidy 2nd, 1904.

In 1918, H. B. Ward (in Ward and Whipple, 1918, p. 408) appended to a description of the genus *Clinostomum* Leidy 1856 the following observation:

Distoma oricola Leidy from the mouth of *Alligator mississippiensis* is undoubtedly a related form as Pratt surmised. It falls in this family, but too little is known of its structure to justify assigning it to a definite genus.

In 1922, E. C. Harrah (1922, p. 66), on the basis of the above assumption placed *Distomum incommodum* (= ? *M. incommodum* = *D. oricola*) under the genus *Clinostomum* species inquirende as follows:

"Species Inquirende.

Clinostomum (?) *incommodum.*
Syn.: *Monostomum incommodum* Leidy 1856.
Distoma oricola Leidy 1884.
Distomum incommodum Leidy 1890.
Monostomum incommodum Leidy 1904."

He dropped Leidy's question mark from in front of ? *Monostomum incommodum* Leidy 1856, and added *Monostomum incommodum* Leidy 1904. The latter being a repetition from the posthumous compilation of Jos. Leidy's contributions to science arranged and edited by his nephew Jos. Leidy 2nd, 1904.

The above is the chronological history of the fluke *Distomum incommodum* Leidy, the original specimens of which I have been unable to locate.

In accepting the construction that ? *Monostomum incommodum* and *Distoma oricola* are identical, as can be seen from the quotations above and according to Leidy who later erected *Distomum incommodum* for them, I cannot agree with Harrah that they are synonymous to *Clinostomum* Leidy 1856. In the same number of the *Proceedings* (Feb. 1856) in which he described ? *M. incommodum*, Leidy gave the generic description of *Clinostomum*. Obviously Leidy would have had no doubt about where to place ? *M. incommodum* if it were a member of that genus. It is, however, a member of the family Clinostomidae as Pratt (1902) and later Ward (1918) pointed out that it would be found to be. I have further evidence that this is so, as I feel certain that I have found the parasite referred to by Leidy as *Distomum incommodum*.

[1] P. 235, J. Leidy 2nd, 1904.

Four specimens of this species were received by Dr J. Percy Moore of the University of Pennsylvania in some material from a Florida collector labelled: *Thorax of Alligator* Mr Edw. F. Stucke, St Petersburg, Florida, March 1927. Prof. Moore requested me to determine the species for the University of Pennsylvania Zoological Department collection. I was happy to examine the specimens and thankful to him for this opportunity to identify them.

The specimens I have are the only ones found since Leidy's time that fulfil the description and the accompanying remarks made by him. The paragraph in the *Proceedings* immediately following Leidy's description of *Distoma oricola*, quoted above, described the appearance of the preserved specimens very accurately. A piece of thoracic material accompanied my specimens and it likewise was marked with circular scars as described by him. The anterior ends of two of my specimens were still fastened to the piece as if boring into the tissue when I received them. As the description of this species is very meagre I am redescribing it here. The attempt to include this form in a systematic treatment of the distomes has necessitated forming a new genus for it, characterised as follows:

Homoscaphis new genus.

Generic diagnosis. Clinostomidae: Large distomes, elongated elliptical, convex dorsally, flat ventrally, unarmed, smooth or wrinkled transversely; posterior end bluntly rounded, sides nearly parallel, lateral margins usually inverted. Oral sucker small, subterminal, usually retracted, dorsal and lateral cuticle may fold over it into reniform collar or lips open ventrally; acetabulum large, globular, conspicuous circular aperture, deep cavity is muscular and square, situated at junction anterior fourth of body. Prepharynx and pharynx lacking. Short oesophagus not evident when oral sucker retracted; bifurcation one-third distance to acetabulum, early part of intestine lies transversely, then turns right angles posteriorly; crura long, narrow, extend into posterior end, provided with minute outpocketings throughout. Excretory canals, lateral, extracaecal, arise from short unbranched stem, pore ventral at caudal tip. Genital pore on ventral genital eminence, median, posterior of forward border of hinder testis, middle of posterior fourth of body; cirrus sac elongated, extends obliquely of long axis of body to opposite forward testis; seminal vesicle voluminous; testes irregularly pyramidal in ventral view, included in posterior third of the body, anterior testis lateral, posterior testis median. Ovary between testes, unlobed, irregular shaped, dorso-lateral of voluminous seminal vesicle; Mehlis gland left of ovary; Laurer's canal present; seminal receptacle absent. Vitellaria largely extracaecal, non-interrupted, extend from level posterior margin of acetabulum to posterior end of body, non-confluent, meet only slightly, posterior to tips of caeca. Uterus consists of a few coils between testes and a large inverted U-shaped expanded loop confined to intercaecal field between ovary and acetabulum, ascending and descending ramus

same diameter, diverticulations lacking, metraterm not especially developed. Eggs thin-shelled, elliptical, embryonated in utero, numerous. Parasites of Reptilia.

Type of the genus: *Homoscaphis incommodum* (Leidy 1856).

It is given its generic name *Homoscaphis* from the Greek combining form *homos*, like, and the noun *skaphis*, a canoe, a skiff, because of its partial resemblance to a small boat.

Clinostomum Leidy 1856 is the type genus of the family Clinostomidae, and because it does not have a prepharynx or pharynx, but does have a retractable oral sucker, a short oesophagus and outpocketings on the intestinal crura, *Homoscaphis* appears closely allied to it. In both *Clinostomum* and *Homoscaphis* the acetabulum is well developed, the ovary is between the testes and the uterus which lies intercaecal between the acetabulum and ovary is inverted U-shaped. The resemblance stops there however. Except for a digestive system similar to the above this new genus has the topography of members of the family Harmostomidae Odhner in which both the genital pore and ovary are between the testes, as in *Glaphyrostomum* Braun for instance, or *Ityogonimus* Lühe (see Fuhrmann, 1928). The disparity between *Clinostomum* and *Homoscaphis* will become quickly evident from the following: The shape and size difference are the first striking dissimilarities. The former are middle-sized distomes with flattened body, as compared with large distomes strongly convex dorsally and flat ventrally (*C. gracile* Leidy 1856 "length to 3 lines"; *C. marginatum* (Rud.) 3·4 mm. in length, as compared to ? *M. incommodum* Leidy "length 9 lines"; = *H. incommodum*, described in this paper, 17·2 mm. in length). The acetabulum of the former is one-third the length of the body from the head end and has a truncated conical aperture with the apex posterior, as compared with the acetabulum one-fourth the length of the body from the head end and a conspicuous circular aperture. The genital pore of the former is just anterior to the anterior testis, as compared with the genital pore ventral of and approximately 100μ posteriad from the forward border of the hinder testis. The testes of the former are tandem in position and just posterior of the equator of the body, as compared with the testes nearly oblique and in the posterior third. The vitellaria are confluent behind the testes in the former, at least in *C. marginatum*, as compared with vitellaria that are non-confluent. The descending arm of the uterus only is inflated in the former, whereas both arms are in the latter. Finally, the outpocketings of the caeca are prominent in the former but are not in the latter. The former is found in fish-eating birds, herring-gulls, bittern, eagle, stork; and fish, such as pike, bass, trout, bluegill, perch, etc., the latter is found in reptiles (alligator). Other genera of this family are known to occur in reptiles too, *Harmotrema* Nicoll 1914 for instance, that occurs in snakes. The above are sufficient differences for the erection of a subfamily. However, no new subfamily name is proposed.

Homoscaphis incommodum (Leidy 1856).

(Figs. 1 and 2.)

Synonyms: ? *Monostomum incommodum* Leidy, 1856, p. 43.
Distoma oricola Leidy, 1884, pp. 47–8.
Distomum incommodum Leidy, 1890, pp. 410–18.

Description. Homoscaphis: The specimens in preservative are grey in colour. Conspicuous features are the incurve of the anterior end; the deep cavity of the acetabulum; the convex dorsal and flat ventral surfaces; outlines of the gravid uterus; the reniform lipped or collared oral sucker; the inverted edges of the body; and finally the prominent genital pore in the middle of the posterior fourth of the body. The entire description as given by Leidy was derived from the above external characteristics.

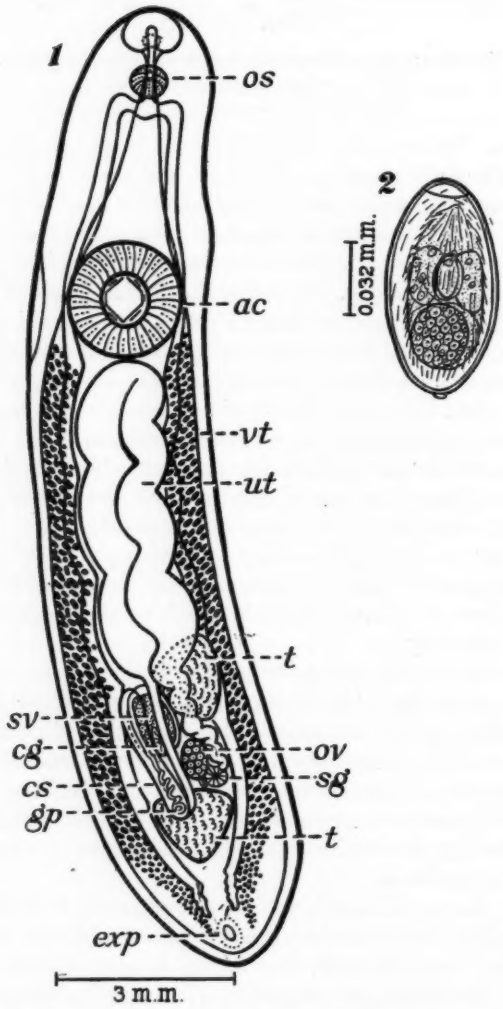
The average length of three adult specimens is 17·2 mm., and the average breadth, measured at the widest part, is 3 mm. The fourth specimen is 14 mm. in length by 3 mm. in breadth. The specimens contain eggs in the uterus and may be regarded as sexually mature adult individuals.

They have an elongated ellipsoidal thick muscular body not transparent and difficult staining. They become moderately wider posteriorly to a width of 3 mm. from after the acetabulum until approximately 2 mm. from the posterior end where they taper roundly to a blunt point. The lateral margins of the anterior, in each case, are incurved ventrally from about the ventral sucker to the forward end and the whole anterior of the worm curves ventrally to the rest of the body.

The cuticular covering and muscular coat is thick and does not show the slightest trace of spines. It is, however, wrinkled transversely from the oral sucker to a point opposite the genital pore. The outline of the gravid uterus is thrown into relief on the convex dorsal and flat ventral surfaces as is also the outline of the acetabulum on the dorsal surface. The lateral margins are inverted in the preserved specimens, as noted by Leidy who employed the word "inverted" in the architectural sense, which gives the body a miniature shallow boat appearance.

The small oral sucker is subterminal and retracted in these specimens so that the dorsal and lateral cuticle rises around and folds over it. The reniform collar, or "lips" quoting Leidy, thus formed is open ventrally and measures 0·418 mm. on the median line and 1·0 mm. in the lateral direction. The linear annulus, mentioned by Leidy as succeeding the reniform lips, is the concentric circles of musculature forming the small oral sucker. This sucker is 0·4–0·45 mm. in diameter. It is smaller than the acetabulum.

The acetabulum is large, spherical or globular in shape and opens ventrally by a conspicuous circular aperture which is deeper than it is wide. It measures 1·54–1·63 mm. in outside diameter; its muscular ring, 365–550 micra in thickness, surrounds an orifice 530–810 micra in diameter. Beneath the surface the cavity is square. The ratio of the ventral sucker to the oral sucker is approxi-



Figs. 1 and 2. *Homoscaphis incommodum* (Leidy 1856).

Fig. 1. Adult specimen. Ventral view.
Fig. 2. Ovum.

Lettering: *ac*, acetabulum; *cg*, compound tubular gland; *cs*, cirrus sac; *exp*, excretory pore; *gp*, genital pore; *os*, oral sucker; *ov*, ovary; *sg*, shell gland; *sv*, seminal vesicle; *t*, testis; *ut*, uterus; *vt*, vitellaria.

mately 4:1. It lies approximately 4·2 mm. from the anterior end measured to the anterior border or about 4·7 mm. from the anterior end measured to the middle, thus it is approximately at the junction of the anterior fourth of the body.

The digestive system has neither prepharynx nor pharynx. There is a very short oesophagus that is difficult to distinguish when the oral sucker is retracted. Bifurcation of the intestine takes place one-third of the distance from the anterior end to the acetabulum. The crura, after a transverse commissure, turn sharply at right angles posteriorly. They appear as simple, long unbranched tubes *in toto* mount. In serial sections small crenations or outpocketings are evident throughout. They pass around the acetabulum, accommodate themselves to the folds of the uterus and extend into the posterior end of the body where their tips come close together. They average 330–360μ in diameter.

The excretory pore is ventral at the caudal tip. A single small stem arises from it. This expands into a small trilobular bladder at the ends of the intestinal crura, which is hardly more than the termini of the canals. Two principal extracaecal lateral canals and a pair of medio-lateral canals arise from this and run the length of the body. Many other branches may readily be seen in serial sections. Retiform anastomosing ramifications of the excretory system are clearly evident throughout the parenchyma in frontal sections.

The genital pore of the reproductive system opens mid-ventrally at the apex of a prominent genital eminence about 2·27 mm. from the posterior end of the body. It is 100 microns posteriad from the forward border of the hinder testis. This position is approximately 1/7·6 of the body length, *i.e.* in the middle of the posterior fourth. An elongated cirrus sac, measuring 327–390 microns in diameter by 1·95 mm. in length, leads from the genital atrium anteriad to a point opposite to the forward testis, recurves and ends in a prominent vesicula seminis externus. This seminal vesicle is 910 microns long by 600 microns in diameter and lies dorsal of the cirrus sac and lateral of the ovary. A coiled cirrus and long coiled ductus ejacularis lies within the cirrus sac. Neither pars prostatica nor prostate glands are especially developed.

The large unlobed testes are irregularly pyramidal in shape in ventral view and nearly oblique in position. The anterior lateral testis has its apex pointed toward the mid-line, the posterior median testis toward the tail end. They are separated by the early coils of the uterus, the Mehlis gland, and by the ovary of the female system. The voluminous vesicula seminis is partially between the testes also. The anterior testis is 1·272 mm. in length by 0·910 mm. in breadth and lies 1·365 mm. in front of the posterior testis. The latter is 1·09 mm. in length by 0·910 mm. in breadth. In transverse sections of a second individual the maximum diameter is 1·18 mm. From the posterior tip of this testis to the caudal end of the body is a distance of 1·575 mm.

The ovary and Mehlis gland are dorso-lateral to the more prominent vesicula seminis and between the testes. The former is unlobed and 636 microns in length by 550 microns in maximum diameter. The Mehlis gland and ootype

are situated to the left of the ovary and half a millimetre anterior of the Laurer's canal which leads off dorsally from the fertilisation space before the ootype. There is a large fertilisation space but no receptaculum seminis evident. The opening of Laurer's canal is 1·815 mm. from the caudal end.

The uterus has several small coils between the testes. Anterior of the lateral testis it broadens immediately into a large loop inverted U-shaped which passes forward as far as the acetabulum and returns to a point opposite to the anterior testis. There it abruptly narrows to the metraterm which leads to the genital atrium. Midway of the metraterm is a compound tubule glandular structure, 270 microns in length by 145 microns in breadth, connected to the duct, whose function is unrecognised. The eight to ten broad folds of the large loop fills all of the space between but do not go beyond the intestinal crura. Distended by numerous eggs the ascending and descending rami slightly overlap one another. They are about the same diameter, varying from 0·82 to 1·25 mm. Diverticulations are absent and the vagina is not especially developed. The numerous thin-shelled elliptical eggs in utero measure as follows: maximum 108 by 48μ; minimum 92 by 44μ; embryonated perfectly elliptical ova 96 by 48μ. They have an opercular cap anteriorly and a tiny knob posteriorly.

Numerous well-developed uninterrupted follicular vitellaria fill the lateral margins from the posterior border of the acetabulum to the posterior end of the body. The greater part lies extracaecal. They are non-confluent but do come very close together posteriorly, just ventral to the tips of the caeca.

Host. Alligator mississippiensis.

Location. Thorax.

Distribution. North America, United States (St Petersburg, Florida).

Type specimens. University of Pennsylvania Zoological Department Collections. Collected by Mr Edw. F. Stucke from a Florida alligator, March 1927.

The following trematoda have been reported from *Alligator mississippiensis* as listed by Stiles and Hassall (1908): *Monostomum incommodum* Leidy; *Distoma oricola* Leidy; *Polycotyle ornata* Will-Suhm; *Diplostomum pseudostomum* (Will-Suhm); and since then *Acanthochasmus diploporus* Stunkard (1931). There may have been others reported but there is no complete host list available.

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THE STRIGEID TREMATODE, *CRASSIPHIALA AMBLOPLITIS* (HUGHES 1927).

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(With 6 Figures in the Text.)

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Introduction.

Within the last decade problems dealing with life cycles and relationships of the Strigeid Trematodes have received considerable attention. Among the European workers Szidat (1929, 1929 *a*) has given the most complete summary of the anatomy, development and systematic position of the Strigeidae, while in North America La Rue (1926), his students and associates, have been contributing to our knowledge of the anatomy, relationships and life histories.

Experimentally demonstrated Strigeid life histories have been published in North America by Van Haitsma (1930, 1931, 1931 *a*), Price (1931) and Bosma (1931). In Europe Szidat (1924, 1924 *a*, 1929 *b*, etc.) has contributed experimentally to our knowledge of the life cycles of this group. During the summer of 1930 an investigation of the life history of the Strigeid metacercaria, *Neascus ambloplitis* Hughes (1927), appearing in the small-mouthed black bass, *Micropterus dolomieu* Lacépède, was undertaken. A brief account of the experiments appears elsewhere (Hunter and Hunter, 1930, 1931). The morphology of the adult is given below with data on the distribution of the metacercaria and a recapitulation of the experiments.

Feeding experiments.

The first step in this study of the developmental cycle of *Neascus ambloplitis* was to secure adult parasites. In June, 1930, six fledgling belted kingfishers, *Streptoceryle alcyon* (L.), were secured; beginning June 25th each of two were fed 250 encysted metacercariae, *Neascus ambloplitis*, from the small-

mouthed black bass, *Micropterus dolomieu* Lacépède (Table I). Three of the other birds were used as controls; all were fed upon the raw flesh of the common sucker or the dwarf sucker, *Catostomus commersonii commersonii* (Lacépède) and *C. commersonii utawana* Mather, respectively. The flesh of these fish was utilised because of its freedom from parasites, particularly

Table I. *Summary of experimental feedings with the belted kingfisher,* Streptoceryle alcyon (*L.*).

	Experimental birds Nos. 1 and 2		Control birds Nos. 3, 4 and 5		
No. of days after June 25th, 1930	Quantity of eggs in droppings	Remarks	Bird No.	Quantity of eggs in droppings	Remarks
0	*N*	Each bird fed 60 encysted metacercariae of *C. ambloplitis*	3, 4, 5	*N*	—
12	*N*	—	4, 5	*N*	—
17	*N*	Each bird fed 100 encysted metacercariae of *C. ambloplitis*	3, 4, 5	*N*	—
22	*N*	—	3, 4, 5	*N*	—
23	*N*	—	3, 4, 5	–	—
25	*N*	—			—
27	+	—	4 (died)	?	77 parasites recovered; 74 = *C. bulboglossa* 3 = *Crassiphiala* sp. Mostly immature
28	–	Each bird fed 50 encysted metacercariae of *C. ambloplitis*	3, 4, 5	–	—
31	–	—	3, 5	+	—
34	–	Each bird fed 20 encysted metacercariae of *C. ambloplitis*	3, 5	–	—
36	+	Each bird fed 20 encysted metacercariae of *C. ambloplitis*	3, 5	+	—
37	–	—	5 (died)	–	42 parasites recovered; 34 = *C. bulboglossa* 8 = *C. ambloplitis*
38	+	—	3	+	—
41	+	—	3 (died)	–	90 parasites recovered; 76 = *C. bulboglossa* 14 = *C. ambloplitis*
44	+ + +	—			—
47	Bird No. 1 (died)	293 parasites recovered; 56 = *C. bulboglossa* 237 = *C. ambloplitis*			—
50	Bird No. 2 (died)	288 parasites recovered; 25 = *C. bulboglossa* 263 = *C. ambloplitis*			—

N = negative; + = a few, less than five eggs per smear; + + + = many, about 25 eggs per smear; – = not examined.

Strigeids. At autopsy 47 and 50 days later 237 and 263 *Crassiphiala ambloplitis* respectively were recovered.

Some idea of the normal infection in wild birds may be secured from the examination of 12 other young and 14 adult kingfishers which revealed a natural mixed infection of *Crassiphiala bulboglossa* and *C. ambloplitis*, the former species predominating in about the same ratio as in the controls. These 12 fledglings were examined within a period of three years from the same locality

that furnished the original group; they showed a minimum infection of one and a maximum of 150 Trematodes of this genus. The average infection for these birds was 38·5 *Crassiphiala* per bird, a number well below our experimental results. In these 21 was the maximum number of *C. ambloplitis* recovered. However, we must assume that a natural infection existed in the experimental birds and endeavour to determine whether or not a significant experimental infection was superimposed upon the one existing at time of capture.

It is unfortunate that other routine work prevented daily faecal smears from the experimental birds and the controls. However, eggs were first detected in the faeces about 27 days after metacercariae were fed to the experimental birds. Twenty-seven days after feeding each of the birds 100 encysted metacercariae of *C. ambloplitis* a significant jump in the egg count was noted. Thus it appears that *C. ambloplitis* requires about 27 ($\pm$ 2 to 3 days), under the conditions described above, to reach sexual maturity. It should also be noted that a period of 31 days elapsed before eggs were recovered from the controls. It is evident that these parasites which were present in the digestive tract at the time of capture must have reached sexual maturity in the controls somewhere between the 22nd and 31st day. If a period of about 30 days is required for *Crassiphiala* to become sexually mature one would not expect to find the last 90 parasites which were fed the birds sexually mature. Every Strigeid Trematode which was recovered had a well-developed hindbody, a structure characteristic of the fully developed adult. But examination of the 237 and 263 *C. ambloplitis* recovered showed that 79 and 83 respectively did not have fully developed eggs in the uterus; this coincides fairly closely with the expected results (cf. Table I).

In the case of the experimental birds a total of 293 and 285 *Crassiphiala* respectively were recovered. These totals are above the 250 fed during the course of the experiment. The obvious explanation lies in the fact that all of the fledglings were infected at the time they were removed from their nest and the large increase came as a result of the experimental feedings. When the 56 and 25 *C. bulboglossa* found in the experimental birds are compared with the 74, 37 and 76 *C. bulboglossa* from the controls it is apparent that such is the case. That a significant experimental infection of *C. ambloplitis* was superimposed upon the "natural" one is shown by the huge increase in the numbers of adult *C. ambloplitis* (see Table I) recovered from experimental birds when compared with the maximum of 14 from the controls. And finally, these findings are supported by the examination of 12 other fledgling kingfishers taken from the same locality in other years.

Recently Krull (1932) reported that he had been able to infect experimentally the common sunfish with *Cercaria bessiae* Cort and Brooks. These cercariae developed into the metacercariae first described as *Neascus ambloplitis* Hughes (1927). As pointed out previously (Hunter and Hunter, 1931) this should now be designated as the metacercaria of *Crassiphiala ambloplitis* (Hughes 1927).

DISTRIBUTION OF THE METACERCARIA.

The metacercaria of *Crassiphiala ambloplitis* was first described by Hughes (1927) from the rock bass and the small-mouthed black bass from Douglas Lake, Michigan. Since 1928 data on the occurrence of this parasite in fish have been gathered from New York State where it has been found also in the large-mouthed black bass, *Aplites salmoides* (Lacépède) (Hunter and Hunter, 1931, 1932).

An analysis of the data shows that 52, or 38 per cent. of 134, adult rock bass examined were infected, while 120, or 36·3 per cent. of 330, and 1, or 1·2 per cent. of 84, of the small- and large-mouthed black bass respectively carried the encysted metacercaria. Fry and fingerlings of the small-mouthed black bass, examined principally from hatchery ponds, carried practically no infection since only 12, or 1·8 per cent. of 633, were infected by this helminth. The infection appears to be confined largely to the larger rock bass and small-mouthed black bass and in these two species may be expected in about 35–40 per cent. of the hosts. Conversely the large-mouthed black bass appears less apt to carry the infection.

MORPHOLOGY OF THE ADULT.

Subsequent study of the anatomy of the adults secured through feeding experiments relegated these parasites to the genus *Crassiphiala* Van Haitsma (1925). A brief morphological description follows:

Specific diagnosis. With characters of genus. Adult possesses smooth cuticula with body constricted into distinct cephalic and caudal regions. Fore-body ranges from 0·48 to 0·62 mm. in length, 0·26–0·29 mm. in width and 0·068–0·071 mm. in depth. Bowl-shaped fore-body contains ventro-terminal oral sucker, acetabulum, and holdfast organ. Oral sucker ranges from 0·084 to 0·12 mm. in maximum diameter; acetabulum is situated in centre of fore-body just anterior to holdfast organ and ranges from 0·044 to 0·052 mm. in length and 0·045–0·056 mm. in width; holdfast organ lies just posteriorly to acetabulum, measures 0·083–0·12 mm. in length and 0·087–0·13 mm. in breadth, bearing a T-shaped opening at apex of rounded, protruding cone. Posteriorly fore-body passes into hind-body which becomes distended dorsally and laterally by uterus and large testes lying in the last two-thirds. Entire hind-body measures 1·36–1·7 mm. in length and 0·28–0·49 mm. dorso-ventrally, while narrow, anterior neck-like region varies from 0·32 to 0·63 mm. in length. Oral sucker opens into well-developed pharynx measuring 0·052–0·063 mm. in length and 0·04–0·045 mm. in breadth; short oesophagus bifurcates into two intestinal crura just anterior to acetabulum; these extend almost to posterior end of body. Large testes situated dorsally in posterior two-thirds of hind-body; anterior testis slightly larger than posterior, measuring about 0·4 by 0·29 mm. compared with latter which is only about 0·33 by 0·29 mm.

in greatest diameter. Each testis drained ventrally (Fig. 1) by a single vas efferens; anterior duct between 0·35 and 0·4 mm. in length, while short posterior one measures less than 0·05 mm. Vas deferens, formed by union of two vasa efferentia, continues posteriorly for about 0·1–0·15 mm. before opening into the seminal vesicle which lies to right of posterior testis, close to the ventral wall, and measures 0·18–0·28 mm. in length by about 0·17 mm. in width. This curves dorsally behind posterior testis, becomes narrower and empties into a muscular ductus ejaculatorius ranging from 0·088 to 0·12 mm. in length and 0·065–0·07 mm. in diameter; this opens into common genital duct about 0·1–0·15 mm. from apex of genital cone. Genital cone measures 0·12–0·13 mm. in length and 0·09–0·11 mm. in width, while genital atrium is 0·07–0·083 mm. in length and 0·18–0·20 mm. in breadth. Small ovary anterior and to left of forward testis measures 0·058–0·091 mm. long and 0·1–0·12 mm. dorso-ventrally. Oviduct arises from posterior median surface of ovary, passes dorsally, keeping to left until reaching middle of anterior testis where it curves ventrally. Laurer's canal arises below peak of curve and opens on dorsal surface midway between the two testes (Fig. 2). Oviduct continues ventrally for 0·17–0·27 mm. before making a **U** turn and passing dorsally into the ootype-Mehlis gland complex. Vitelline reservoir lies adjacent to Mehlis gland and empties into proximal end of ootype by means of a short duct (Fig. 2). This reservoir, lying between the testes, measures about 0·04 mm. long by 0·06 mm. wide and 0·1 mm. in depth and is formed by the union of left and right vitelline ducts; latter is shorter, having its origin midway between ventral and dorsal walls about the level of anterior testis; left vitelline duct leaves vitellaria close to anterior testis on opposite side. Lateral and ventral vitellaria extend from anterior level of the genital atrium to base of neck of hind-body (Figs. 1, 6 A). Seminal receptacle absent. Uterus arises from distal extremity of ootype and continues dorsally between testes until close to dorsal wall where it dips underneath anterior testis, continues anteriorly to the base of neck-like

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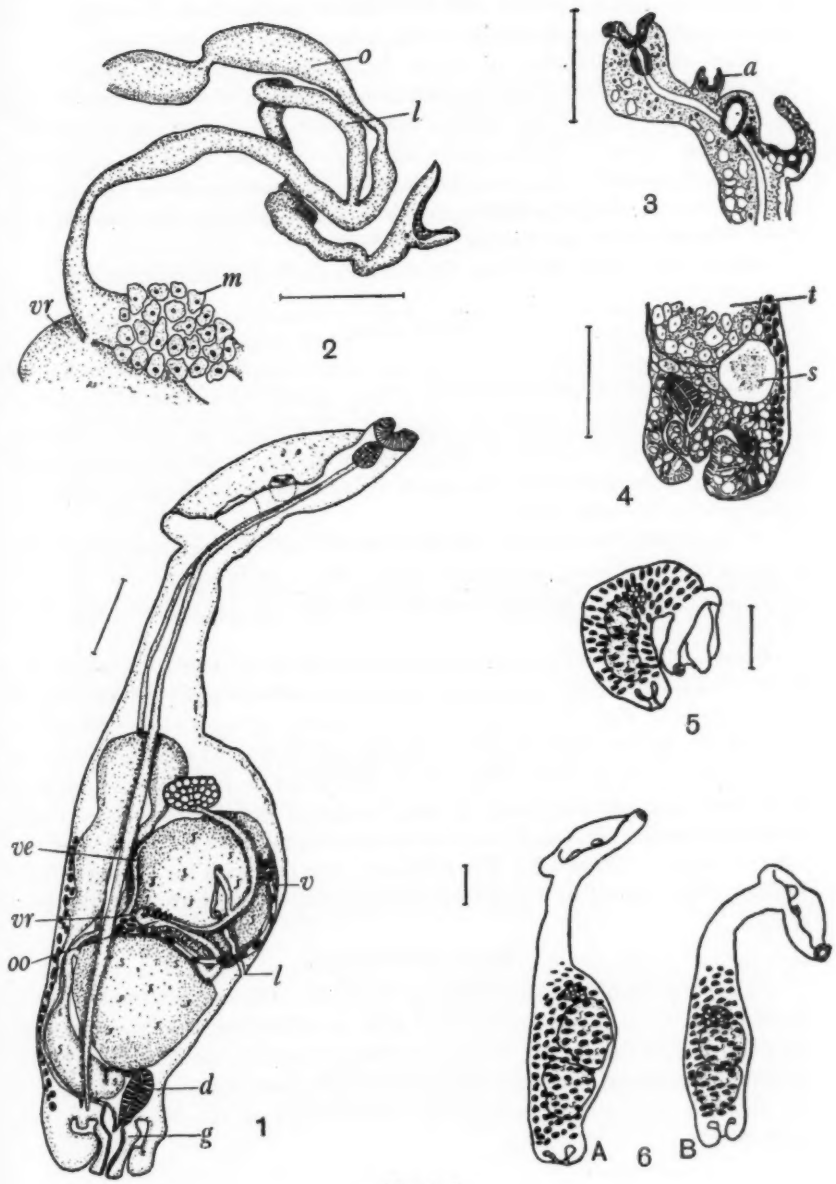
Legends to Text-figs. 1–6.

Fig. 1. *Crassiphiala ambloplitis.* Reconstruction to scale of the adult.
Fig. 2. Reconstruction to scale of a part of the female reproductive system.
Fig. 3. Composite drawing of eight sections through anterior end.
Fig. 4. Sagittal section through posterior end.
Fig. 5. *C. bulboglossa* Van Haitsma 1925.
Fig. 6 A and B. *C. ambloplitis* showing characteristic shapes.

All lines have the value of 0·2 mm.; all drawings were made with the aid of a camera lucida except Figs. 1 and 2.

Abbreviations.

a, acetabulum.
d, ductus ejaculatorius.
g, genital cone.
l, Laurer's canal.
m, Mehlis gland.
o, oviduct.
oo, ootype.
s, seminal vesicle.
t, testis.
v, vitelline duct.
ve, vas efferens.
vr, vitelline reservoir.



Figs. 1–6.

region before looping posteriorly; distal part, distended and filled with spermatozoa, follows ventral wall to common genital duct. The eggs vary from 90 to 99 μ in length and 56 to 66 μ in breadth.

Host. Adult: intestine of belted kingfisher, *Streptoceryle alcyon* (L.). *Metacercaria:* encysted under the scales and between the myotomes of the small-mouthed black bass, *Micropterus dolomieu* Lacépède; the rock bass, *Ambloplites rupestris* (Rafinesque); the large-mouthed black bass, *Aplites salmoides* (Lacépède); the common sunfish, *Eupomotis gibbosus* (L.) (Krull, 1932). *Cercaria:* found in *Heliosoma trivolvis* (Say) where it was described as *Cercaria bessiae* Cort and Brooks (Krull, 1932).

Slides of the adult have been deposited in the U.S. National Museum.

Discussion.

Crassiphiala ambloplitis clearly belongs in the Strigeidae and the subfamily Polycotylinae Monticelli (1892) for it has definite fore- and hind-bodies; a flattened fore-body, a holdfast organ which is circular, bulbous or elliptical and penetrated by a cavity; no lateral suctorial cups or ear-like appendages, nor cirrous pouch and cirrus, the uterus being confined to the hind-body and opening at its posterior end.

C. ambloplitis fits in Van Haitsma's genus with a single exception, the presence of a functional acetabulum rather than a rudimentary one. In view of this discrepancy the genus is emended by Bosma (MS.) to include forms with an acetabulum.

C. ambloplitis differs from *C. bulboglossa* in possessing a definite acetabulum, definite neck-like region, a muscular ductus ejaculatorius and a well-developed genital cone (cf. Figs. 5, 6 A and B). Furthermore the eggs of *C. ambloplitis* are typically smaller than those of *C. bulboglossa*. Preserved specimens may be easily separated by the body shape. In *C. bulboglossa* the fore-body is normally bent back upon the hind-body so that the dorsal surfaces of each part are almost contiguous while in *C. ambloplitis* the longitudinal axis is only slightly bent (cf. Figs. 5, 6 A and B). This difference, together with the thin neck-like region, clearly furnishes a macroscopic basis for the separation of the two species.

Acknowledgments.

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LIFE HISTORY STUDIES ON TREMATODES OF THE SUBFAMILY RENIFERINAE[1].

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(With 24 Figures in the Text.)

Introduction.

The members of the subfamily Reniferinae form a rather compact group of Trematodes which are easily distinguishable, both in structure and type of life history, from the rest of the forms included in the family Plagiorchidae. Most of the studies of the Reniferinae have been concerned with the description of new species, and only recently has any attention been directed toward the solution of their life history. The first significant facts which led to the completion of the present life histories were contributed by McCoy (1928). McCoy infected snails with the ova collected from species of three genera of the Reniferinae, viz. *Dasymetra conferta* Nicoll 1911, *Pneumatophilus variabilis* (Leidy) Odhner 1910, and *Renifer kansensis* Crow 1913, and obtained in these snails the mature cercaria of each species. In addition to determining experimentally the cercaria of these forms, he was able to demonstrate that these cercariae penetrate and form normal cysts in tadpoles which when fed to the snake would establish an infection in this host. These observations, therefore, proved that the life histories of species of three genera of this subfamily include three hosts: the snail, the first intermediate host; the tadpole, the second intermediate host; and the snake as the definitive host.

In discussing the structure of the cercariae which he studied, McCoy pointed out that they are practically morphologically indistinguishable, each having the same Y-shaped type of excretory bladder with lateral arms extending anterior to the acetabulum. The close similarity in structure of these cercariae led him to postulate that other members of the subfamily Reniferinae should have cercariae of this type.

Very recently Ingles (1933) has completed the life history of *Zeugorchis syntomentra* Sumwalt 1925, also a member of the Reniferinae. The cercaria of

[1] Contribution from the Biologica /Station of the University of Michigan and the Department of Helminthology of the School of Hygiene and Public Health of the Johns Hopkins University. I wish to take this opportunity to express my appreciation to Dr W. W. Cort, under whose direction these studies were made, for his many helpful suggestions and criticisms. I am also indebted to Dr G. R. La Rue, University of Michigan, for courtesies extended during the progress of the experimental phase of the studies at the Biological Station of the University of Michigan.

this form is of the same general type as described by McCoy for this subfamily, and its life history includes snails of the same genus, as well as the tadpole as the second intermediate host.

The present paper deals with the life histories of three Trematodes of the subfamily Reniferinae, one previously described, *Lechriorchis primus* Stafford 1905, and two new forms, one also a member of the genus *Lechriorchis* and the other belonging to a new genus.

MATERIALS AND METHODS.

The following genera and species of snakes were used in the life-history studies: *Thamnophis sirtalis*, *T. sauritus*, *Natrix sipedon* and *N. septemvittata*. These snakes were collected in West Virginia in the early spring and shipped to the Biological Station of the University of Michigan. Since all snakes examined from the West Virginia area were found to have a very low incidence of trematode infestation, they were considered suitable for experimental hosts. One five-month-old laboratory-raised snake, *Thamnophis sauritus*, was used for one infection experiment in the studies on the life history of *Lechriorchis primus*.

Sardines were found to be an excellent cheap snake food, and after the snake becomes accustomed to its cage, it will readily take this food directly from the forceps. However, it was found that the snake would invariably refuse to eat sardines packed in cotton-seed oil. During the winter months, the snakes were fed about once a month, but in the spring, when they were emerging from their lethargic condition, once a week was not considered too often. For very young snakes, bits of French fillets were used as food, since it was frequently necessary to force them to eat the first two or three times.

The fumes of xylol serve as an excellent anaesthetic in killing the snakes for autopsy, because the Trematodes are not affected by these fumes and the snake host is perfectly relaxed and can very readily be dissected. Care should be taken not to have an excess of xylol in the killing chamber in order to prevent aspiration of the fluid into the lungs, since the Trematodes are quickly killed when they come in contact with the xylol.

Laboratory-raised snails, *Physella*[1] *gyrina* (Say), were used for all sporocyst studies. To obtain cercariae for infection purposes, *P. gyrina* (Say) and *P. parkeri* (Currier) were collected from Grape Vine Point on Douglas Lake, University of Michigan Biological Station, an area where the incidence of cercariae of the Reniferinae type in snails is exceedingly low.

In the life-history studies of *Lechriorchis primus* Stafford 1905, *Caudorchis eurinus* n.gen. et sp., the tadpoles to be used as experimental hosts, larval forms of *Rana pipiens* and *R. clamitans*, were collected from Vincent Lake, a small

[1] All generic and specific names of snails used in this paper are in accordance with the nomenclatorial changes proposed by F. C. Baker (1928), in a monograph on the fresh water mollusca of Wisconsin. Baker has shown that the American species of *Physa* have certain distinct differences from the European type *Physa fontinalis*, particularly in the mantle and the number of denticulations on each side of the centre cusp. In view of these differences, the genus *Physella* Haldeman 1842 is redefined to nclude the American species of *Physa*.

acid lake in the vicinity of Douglas Lake. The fauna of Vincent Lake has been studied by Jewell and Brown (1924, 1929) and by other investigators over a period of several years and none of them has ever reported finding snails. Therefore, the absence of snails from this lake permits the assumption verified by repeated examination that tadpoles living in this lake are free from trematode larval stages. The tadpole intermediate hosts for the studies on the life history of *Lechriorchis tygarti* n.sp. were collected from a small acid pond on the campus of Davis-Elkins College, West Virginia, which was known to be free from snails.

All detailed morphological studies were made on living Trematodes, but measurements and drawings were made of specimens fixed in hot Gilson's sublimate-acetic solution, stained in Ehrlich's acid haematoxylin, and counterstained with eosin. It was found that living Trematodes stained with neutral red and mounted in glycerine could be studied in this condition for a considerable period of time. Since it is rather difficult to study the ovarian complex in fixed material, this method was found to be valuable in working out the details of this structure.

Morphological studies were made only on living miracidia, but specimens to be measured were fixed according to the method outlined in the section on the miracidia. Certain *intra vitam* stains were found to be of great value in emphasising structures in the cercaria which are partially obscured by the cystogenous glands. For instance, brilliant cresyl blue and Bismarck brown stain the penetration glands a deeper hue in contrast with the rest of the body. Cercariae taken from the mature daughter sporocyst were found to be better material for the study of the flame cell pattern. Before the cercaria has escaped from the sporocyst, the collecting ducts of the excretory bladder are greatly dilated and their course can be more readily followed. To obtain uniform specimens for measurements, cercariae were killed with heat by pressing the worm under a coverslip and drawing the slide slowly through a flame.

The life history of *Lechriorchis primus* Stafford 1905.

Experimental evidence.

After preliminary observations which determined the cercaria and general course of life history of *Lechriorchis primus*, a series of experiments was conducted in order to complete the details of the life cycle including a careful morphological study of all stages. The cercaria of *L. primus* was experimentally obtained by infecting snails with the trematode ova, and the cercaria thus obtained was used in infecting tadpoles which were subsequently fed to the snake host.

One hundred snails, *Physella parkeri*, that had been kept in the laboratory for four weeks were infected with the ova of *Lechriorchis primus*, found as a natural infection in garter snakes collected near the Biological Station of the University of Michigan. Snails from this lot were examined from time to time and various stages of mother and daughter sporocysts were found, but mature

cercariae were not discovered until the end of the fourth week of infection. At the end of the fifth week of infection all the remaining snails were producing cercariae.

Full-grown tadpoles of *Rana clamitans* and *R. pipiens* were heavily infected with the experimental cercariae. In order to have some degree of uniformity of infection, no more than four tadpoles were exposed to the twenty-four hour cercaria output of a single snail. The metacercariae were then allowed to mature for a week before the tadpoles were fed to the snake host. Twenty snakes, ranging from 10 to 14 in. in length and consisting of two genera and three species, viz. *Natrix sipedon*, *Thamnophis sauritus*, and *T. sirtalis*, were isolated in individual snake boxes and each was fed two infected tadpoles of approximately equal size. In order to eliminate any possibility of infection from food sources, the snakes were fed either tadpoles collected from Vincent Lake or sardines. Table I presents an outline of the autopsy schedule of the snake hosts

Table I. *Outline of data from snake hosts experimentally infected with* Lechriorchis primus.

Host	Age of infestation in months	No. of worms recovered	Position in hos
1. *T. sauritus*	2½	2	Small intestine
2. *N. sipedon*	2½	0	—
3. *T. sauritus*	3	0	—
4. *T. sauritus*	4	70	Small intestine
5. *T. sauritus*	4	60	,,
6. *T. sirtalis*	4	2	,,
7. *T. sirtalis*	4	50	,,
8. *T. sauritus*	4½	0	—
9. *T. sauritus*	4½	197	Small intestine
10. *N. sipedon*	5	0	—
11. *N. sipedon*	5½	0	—
12. *T. sauritus*	6½	90	Stomach and small intestine
13. *T. sirtalis*	7	60	Oesophagus
14. *T. sauritus*	8½	50	Upper oesophagus
15. *T. sauritus*	8½	254	Small intestine
16. *T. sauritus*	10	20	Upper oesophagus
17. *T. sauritus*	11	35	First part of lung
18. *T. sirtalis*	12	25	,,
19. *T. sirtalis*	12	20	,,
20. *T. sauritus*	12½	562	Oesophagus

of this group together with the number of worms recovered and the seat of infection at the time of the autopsy. According to this schedule, snakes were autopsied at regular intervals over a period of twelve months, and practically every snake was found to be infected and the various stages of migration of the young Trematodes from the small intestine to the lung were obtained. During the four summers that these studies were being conducted, 145 garter snakes were collected in the vicinity of the Biological Station and were examined for trematode parasites. Of this number of snakes examined, thirty-one were found to be infested with a total of 359 specimens of *Lechriorchis primus*. On the basis of these figures, the percentage of infestation in nature in this region is approximately 25 per cent. and in comparison 75 per cent. of the twenty

experimental hosts were experimentally infested. In three of the cases in which negative results were obtained (Hosts 2, 10, 11, Table I) it might be considered that these snakes were not experimentally infested because *Natrix sipedon* is not a favourable host. *N. sipedon* has been found to be naturally infested with *Lechriorchis primus*, but never more than three worms have been found in a single specimen of this host.

A final experiment with laboratory-raised hosts.

Fifty laboratory-bred snails, *Physella gyrina*, approximately five months of age, were infested with the ova of *Lechriorchis primus*. At the end of the fifth week all the snails were producing cercariae. Small tadpoles of *Rana clamitans* were given a rather light infection with the cercariae, since the snake to be infected was quite young and would not be able to endure a very heavy infestation. The laboratory-bred snake was then fed five of the infected tadpoles at the first feeding and two a week later. For the remaining time until it was autopsied, it was fed earthworms and bits of French fillets. Five months after the initial feeding, the snake was autopsied and thirty immature worms were recovered from the lower part of the oesophagus. While these worms were not sexually mature, it was quite evident that they were growing and developing in a normal host. Furthermore, they were identical with the worms recovered in Host 13, Table I, and it is concluded that this experiment not only substantiates the observations made in the previous studies, but also presents conclusive evidence for the experimental life history.

Description of the life-history stages of *Lechriorchis primus*.

General course of the life cycle.

The adult worm of *Lechriorchis primus* inhabits the posterior membranous part of the lung of *Thamnophis sirtalis* or *T. sauritus*. The ova are deposited in the lung and are apparently conveyed to the mouth by the ciliary action of the respiratory epithelium, swallowed and passed out with the faeces of the snake. The ova then are ingested by the snail intermediate host, *Physella gyrina* or *P. parkeri*, and hatch in the stomach. The miracidium actively penetrates the stomach wall and comes to rest along the surface of the digestive tract where metamorphosis into the mother sporocyst occurs. Three weeks after infection, daughter sporocysts escape from the mother sporocyst and enter the lymph spaces of the liver of the snail. The daughter sporocyst matures and produces cercariae within from one to two weeks after it reaches the liver. The cercariae escape from the snail, penetrate the tadpole intermediate host and encyst in the body musculature. If the tadpole is then eaten by the snake, definitive host, metacercariae excyst in the stomach and pass to the small intestine. The young worms remain in the small intestine for a period of about seven months, after which they migrate through the stomach to the oesophagus. Here they remain for about three months and at the end of from ten to twelve

months after infection, they pass through the mouth and enter the lung via the trachea. The young worms, therefore, reach the lung in the late spring or early summer. They continue to grow in size, but do not become sexually mature until the second summer and the maximum size is probably not attained until the third year after the initial infection.

The egg and miracidium.

The eggs of *Lechriorchis primus* contain fully developed miracidia when deposited in the lung of the snake. They are oval, operculate, have a thin, dark brown and elastic shell, and measure 48–50 × 23–25μ *in utero*. In the end opposite the operculum, there is a single large yolk cell which may well be used as a criterion in the identification of the ova of other members of the Reniferinae, since the species of six genera which have been studied all have ova with this characteristic.

The eggs appear to be conveyed to the mouth of the snake by the ciliary action of the epithelium of the lung and trachea and are then swallowed and passed out with the faeces. The examination of heavily infected snakes revealed many ova in the trachea, oesophagus, and faecal content which would tend to verify this assumption.

While the eggs are ready to hatch when expelled from the snake, they will not hatch in the open, but only when they are ingested by the snail host or come in contact with the snail gastric juices. It was found that the ova would hatch in the gastric juices drawn from five species of *Physella*, viz. *P. ancillaria* (Say), *P. sayii* (Tappan), *P. magnalacustris* (Walker), *P. heterostropha* (Say), and *P. parkeri* (Currier). This gastric action appears to be specific to species of *Physella*, as the ova of this trematode failed to hatch in gastric juices taken from the following snails: *Heliosoma trivolvis winslowi* (Baker), *H. campanulatus* (Say), *Lymnea stagnalis perampla* (Walker), *Stagnicola emarginata angulata* (Sowerby), and *Bulimnea megasoma* (Say).

Within an hour after the ova are ingested by the snails, hatching takes place. First the flame cells of the miracidium begin to beat and there is a slight contraction and relaxation of the body musculature. Shortly afterwards the body cilia begin to vibrate and the miracidium turns over and over, pushing against either end of the shell in an attempt to escape. Just as soon as there is the slightest break in the operculum the anterior end is thrust out, and after several contractions the miracidium is free in the gastric contents of the stomach. Finding itself free in the proper intermediate host, it now seeks that portion of the snail tissue to which it will become permanently attached.

A living miracidium of *Lechriorchis primus*, when not in an active state of contraction and expansion, assumes a somewhat acuminate shape. However, when relaxed with a fixing agent, it is rather ovoid (Fig. 1). Ten specimens killed with a hot solution of 1 per cent. formalin introduced with a capillary pipette at the edge of the coverslip measured on an average 45 × 19μ. The entire surface of the body, with the exception of the anterior tip, is covered

with rather long cilia which measure about one-sixth of the width of the worm. They are equally distributed over the body and there are no non-ciliated areas as described for the miracidium of *Schistosomatium douthitti* (Price 1931). The penetration apparatus consists of an anterior papilla (Fig. 1, *ap*) at the base of which there are two openings for the penetration glands. The two large granular penetration glands (*pg*) occupy most of the body cavity and overlap to about their lateral one-third. The central nerve mass (*sp*) is bilobed, each lobe occupying an indentation in the penetration glands at the base of their ducts. There are two flame cells (*fc*) in the extreme posterior end of the body each provided with a slightly convoluted excretory duct, which opens separately by the lateral excretory pores. The excretory pores are situated in the anterior third of the body and on a line with the posterior extremity of the primitive gut. The primitive gut (*gs*), which is completely closed, extends posteriorly from the anterior papilla, at first as a slender tube between the two ducts of the penetration glands to immediately expand into a large sac. The miracidium has a surprisingly small number of germ cells (*gc*), usually four, which are quite large and float free in the body cavity.

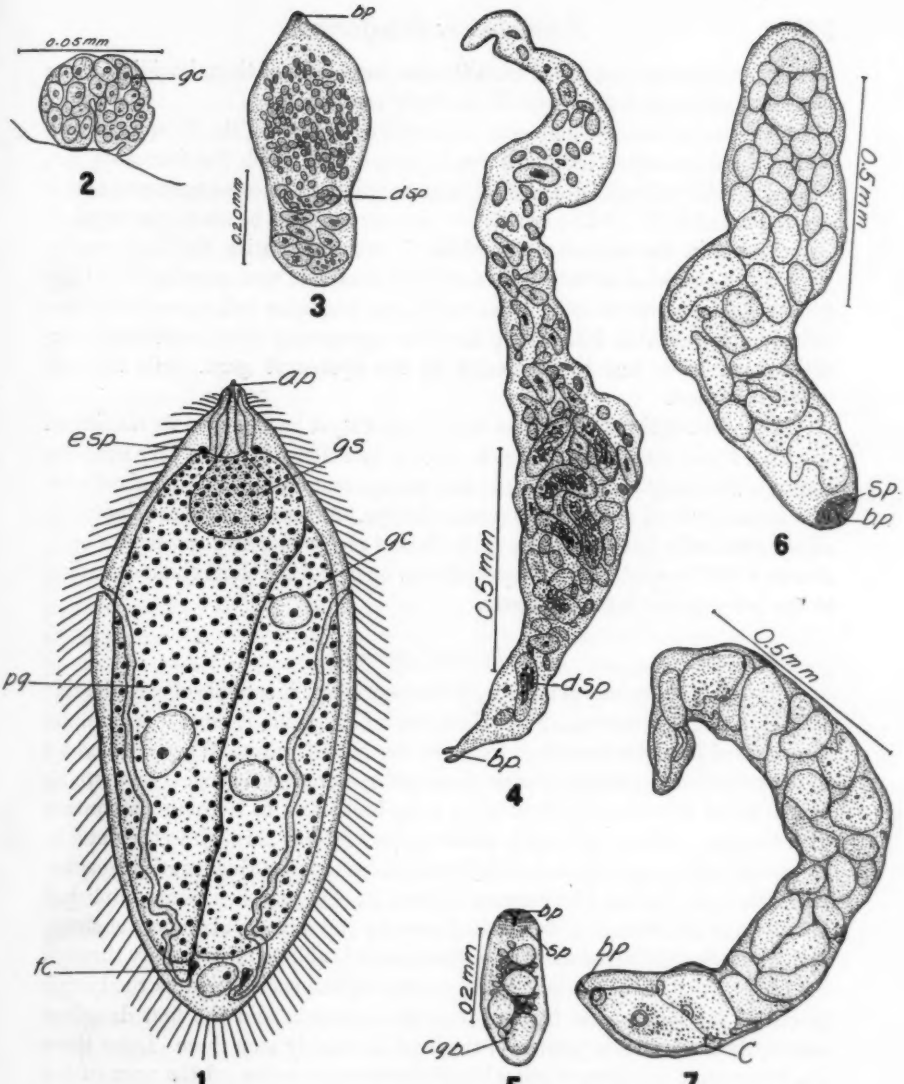
The miracidium of *Lechriorchis primus* is not an active swimmer even though it is equipped with well-developed cilia, rather it worms its way through the gastric mass of the snail to the wall of the stomach where it actively penetrates through the mucosa to the body cavity of the snail host. The evidence seems to be very conclusive that the miracidia are not expelled with the faeces of the snail to penetrate subsequently from the outside, but always penetrate through the wall of the stomach, since miracidia were never found in the dish where snails were freely feeding upon the trematode ova, nor were they found in faeces taken from the intestine of the snail.

After the miracidium has penetrated the stomach wall, it usually becomes attached to the outer wall of the stomach or first part of the intestine. In this position it metamorphoses into the mother sporocyst.

The mother sporocyst[1].

The very young mother sporocyst is more readily found in young snails. The visceral mass of snails of the genus *Physella* of approximately five months of age can be mounted on a slide and studied with both the low and high powers of the compound microscope. The youngest mother sporocysts studied were obtained one week after the snails had been exposed to the trematode ova. The mother sporocyst (Fig. 2), at this stage of infection, measures $62 \times 16\mu$, a comparatively slight increase in size over the miracidium. The wall of this sporocyst is composed of a high cuboidal epithelium one layer in thickness in the body proper, but the anterior end is composed of from one to two layers of somewhat smaller cells. The most significant change is in the number of germ

[1] A description of the structure of the mother sporocyst of *Lechriorchis primus* Stafford has been published in the Proceedings of the Helminthological Society of Washington, *J. of Parasitology*, **14**, 250 (1933).



All drawings, with the exception of Fig. 1, were made with the aid of the camera lucida.

Figs. 1–7. *Lechriorchis primus*, larval stages in the snail host.

Fig. 1. Miracidium showing germ cells, penetration glands, primitive gut, and flame cells.
Fig. 2. Young mother sporocyst one week after infection containing undifferentiated germ cells.
Fig. 3. Mother sporocyst two weeks after infection, containing young daughter sporocysts.
Fig. 4. Fully mature mother sporocyst, three weeks after infection.
Fig. 5. Young daughter sporocyst just after escape, containing cercaria germ balls.
Fig. 6. Daughter sporocyst from liver of snail, four weeks after infection.
Fig. 7. Daughter sporocyst containing fully mature cercariae, five weeks after infection.

Lettering: *ap* = apical papilla; *bp* = birth pore; *c* = cercaria; *cgb* = cercaria germ ball; *dsp* = daughter sporocyst; *esp* = eye spot; *fc* = flame cell; *gc* = germ cell; *gs* = primitive gut; *pg* = penetration gland; *sp* = spines.

cells (*gc*) which have increased from the four large cells of the miracidium stage to a number which completely fill the body cavity.

After two weeks development, the mother sporocyst (Fig. 3) appears as a whitish nodule, somewhat pyriform in shape and which has increased sufficiently in size to be studied at 20 × magnification. A selected specimen of this age measured 0·61 × 0·28 mm. The birth pore (*bp*) can be seen as a terminal indentation in the anterior end which is connected with the body cavity by a short occluded channel. In a sporocyst of this age, practically all the germ cells have formed germ balls and in the posterior end, germ balls have already differentiated into young daughter sporocysts (*dsp*) containing cercaria germ balls, but the majority of the sporocyst germ balls are still undifferentiated.

At the end of three weeks, the mother sporocyst has reached its maximum size (Fig. 4) and measures 1·7 mm. in length. It is elongate with a very irregular contour. The body wall is very thin and transparent and the epithelial cells are now found to be of a very flat squamosal type. At this stage of development, all the germ cells have formed germ balls and many daughter sporocysts have matured and escaped. The birth pore is now open and the short channel leading to the body cavity is spread apart.

The daughter sporocyst.

The daughter sporocyst is a very active worm and is capable of considerable extension and contraction. Since there are no body cilia it must depend upon this method to make its escape from the mother sporocyst. When a daughter sporocyst reaches maturity, inside the mother sporocyst, it begins to worm its way through the mass of developing germ balls, and as it comes in contact with the body wall an attempt is made to force its way through to the outside. However, not being able to do this, it continues moving about until it reaches the birth pore channel where egress is possible. Escape, then, is accomplished by the sporocyst thrusting its spinated anterior end into the birth pore channel, and by a series of extensions and contractions it is able to force its way through the birth pore. Once free in the body cavity of the snail, it enters the lymph spaces of the lobe of the liver nearest the stomach, and the first daughter sporocysts to enter this portion of the liver invariably stop there. Later there is a progressive invasion of other daughter sporocysts toward the apex of the liver, and those that entered the liver first are producing cercariae when the entire liver is infected.

The daughter sporocyst at the time of its escape from the mother sporocyst (Fig. 5), about three weeks after infection, measures 0·35 × 0·14 mm. It is somewhat oval and the anterior one-third of the body is covered with tiny spines. The body wall is composed of a single layer of cuboidal cells with the exception of the anterior end where the epithelial cells are from two to three layers thick. The terminal birth pore is connected with the body cavity by a short channel which, in the young daughter sporocyst, is completely occluded.

Four weeks after the initial infection, the daughter sporocyst (Fig. 6) is well established in the lymph spaces of the liver and a selected specimen measures 0·95 × 0·17 mm. The body cavity is full of cercaria germ balls of unequal size, and a few of the germ balls have begun to differentiate into cercariae. In a daughter sporocyst of this age, the birth pore (*bp*) is quite prominent and its channel is slightly spread apart.

During the fifth week of infection, the daughter sporocyst reaches its maximum size and the first cercariae are given off. A selected specimen (Fig. 7) measures 2·2 mm. in length. Its wall is very thin and transparent and the epithelial cells are greatly flattened. Within the sporocyst, many full-grown cercariae can be seen vigorously moving about, but the majority of germ balls are as yet undifferentiated.

The escape of the cercaria.

When the cercaria reaches maturity, it becomes quite active and begins to move about in the sporocyst lashing its tail from side to side. Its movement, however, does not seem to be directed toward any particular part of the sporocyst, but just as soon as it comes in contact with the sporocyst wall, it attempts to force its way through without success. But when the cercaria chances to reach the anterior end of the sporocyst, it finds that the birth-pore channel can be readily spread apart by the slightest force and the anterior end of the body protruded out to the birth pore. Then by a series of extensions and contractions and by the aid of the body spines, the cercaria is able to free itself into the distended lymph spaces of the liver and eventually escapes to the outside by following the course of the lymph sinuses of the snail host.

The morphology of the cercaria.

The cercaria of *Lechriorchis primus* (Fig. 8) is elongate, broader at the posterior end. The tail is almost as long as the body and is attached to the posterior end in a rather deep groove. The entire body is covered with tiny spines, which are more numerous and larger between the ventral sucker and the anterior end. The outer rim of the acetabulum is equipped with five rows of particularly large spines. Measurements were obtained from fifty fixed specimens, and all measurements mentioned in connection with this discussion represent the average for this number. The body measures 0·475 × 0·171 mm. The oral sucker has a diameter of 0·072 mm. It is oval and subterminal in position. The acetabulum, which is located just back of the middle of the body, has a diameter of 0·085 mm. The stylet, which is slender with rather weak shoulders, measures 18μ in length by 4·5μ in width at its base. This cercaria possesses two groups of six penetration glands, clustered about the caeca of the intestine. The ducts of each group converge into two simple tubes which open at the base of the stylet. The oral cavity is followed by a short prepharynx and a small pharynx which is usually obscured by the many pharyngeal glands. The short narrow oesophagus divides midway between the pharynx and aceta-

bulum into two short intestinal caeca which only slightly overlap the arms of the excretory bladder. The excretory bladder has a long slender stem, which extends to the posterior limit of the acetabulum, and two lateral arms which almost completely encircle the acetabulum. The flame cell pattern of this cercaria (Fig. 8) is of the "2 × 3 × 6" type.

Biology of the cercaria.

The cercaria of *Lechriorchis primus* always escapes from the snail host at night and usually within two hours after dark, but a few will escape during the day if the snails are placed in a dark chamber. The output of a single naturally infected snail was determined for twenty-four-hour intervals over a period of two weeks, in order to determine the number of cercariae which would be available from a single infection, to infect the tadpole host. After each twenty-four-hour interval, the cercariae which had escaped were killed in 5 per cent. formalin solution and a direct count made. The temperature of the water in which the snail was kept was also taken at the time each count was made, in order to ascertain if variations in temperature could be correlated with the number of cercariae to escape over the period in which the studies were made. The results of these observations are presented in Table II.

Table II. *The number of cercariae of* Lechriorchis primus *to escape from a single snail,* Physella parkeri, *over a period of two weeks.*

Days	No. of cercariae	Temp. of water ° C.	Days	No. of cercariae	Temp. of water ° C.
1	1733	19	8	549	14
2	2624	23	9	283	18
3	2213	26	10	135	14
4	1305	20	11	10	16
5	386	17	12	20	15
6	417	15	13	10	17
7	521	14	14	6	18

When the data in Table II are analysed, it is found that there is a rapid decrease in the number of cercariae given off following a few days of maximum output. Similar observations were made on fifty experimentally infected laboratory-bred snails, *Physella gyrina*, the maximum output never extending beyond a period of five days, although a few cercariae were given off daily for as long as a month. It is probable, therefore, that variations in temperature of the type in Table II would not be an important factor in determining the number of cercariae to escape at any one time, but that most of the cercaria germ balls mature at about the same time.

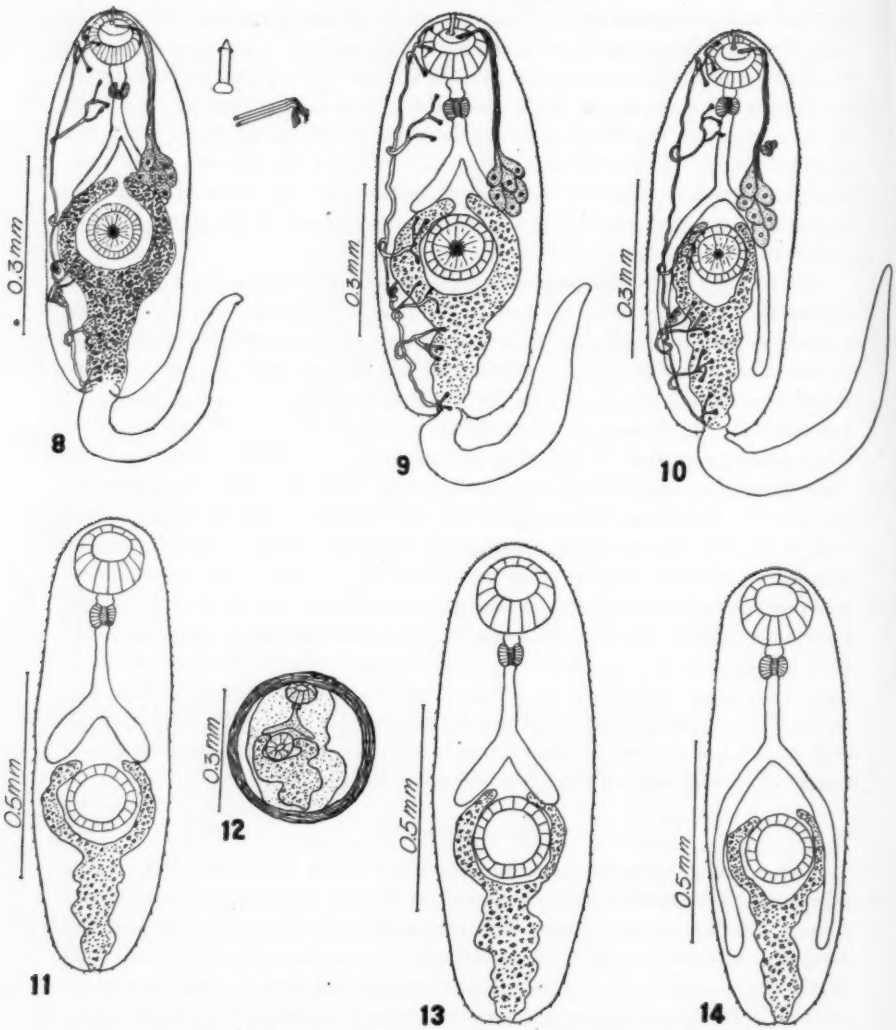
Light does not seem to be an important factor in determining the direction in which the cercariae will swim. This fact was demonstrated when cercariae were exposed to different intensities of light by placing them under a condition in which the quantity of light could be regulated. This condition was obtained by putting the cercariae in a finger bowl one-half the bottom of which was

covered with an opaque sheet of paper, and by directing a strong light on the uncovered portion, a dark and light area could be effected. After the water in the finger bowl had become perfectly still, it was observed that cercariae were not concentrated in any particular area of the bottom, but there seemed to be about as many on the bright as on the dark side. Further, when the water was agitated a little, the cercariae would swim within a short distance of the top, relax and gradually sink to the bottom without any apparent attraction to light. Here again there were about as many on one side of the finger bowl as on the other.

The finding of the tadpole intermediate host appears to be a matter of chance rather than by any chemotropic reaction. This was tested by placing a small tadpole in a shell vial, the mouth of which was covered by the thickness of cheese cloth, which was then submerged into a finger bowl of water containing the cercariae and secured in the horizontal position so that it would be necessary for the cercariae to enter from the lateral position. When the water was agitated, cercariae would swim toward the surface past the cheese cloth without making any attempt to enter the vial. This experiment was then repeated, but in this case fresh tadpole skin was stretched over the mouth of the vial. When the vial was in the horizontal position, cercariae would pass the skin without any attempt to penetrate it, but in the vertical position where it was possible for the cercariae to fall upon the skin, active penetration began immediately. The fact that the cercariae have been observed trying to penetrate the bottom of the finger bowl would further indicate that the finding of the intermediate host is purely a matter of chance. Consequently these cercariae will penetrate many small vertebrates such as larvae of *Triturus*, perch and sunfish, but in these animals the metacercariae do not grow and within the course of a week most of the cysts will have died.

The metacercaria.

The cercaria, upon entrance into the body of the tadpole host, forces its way into the connective tissue between the muscle bundles and immediately encysts forming at first a very thin cyst wall easily broken by the slightest pressure. In this early stage of encystment, the animal floats free in the fluid within the cyst. The cyst is practically spherical, and a few hours after encystment the average diameter for ten cysts was 0·156 mm. The metacercaria (Fig. 11) continues to grow for a period of about a week and the diameter of the cyst increases as successive layers are added to its wall until it takes on a laminated appearance with dark brown pigmentation. The mature cyst (Fig. 12), five days after encystment, has an average diameter for ten cysts of 0·357 mm. Cysts taken from the muscles of a naturally infected adult frog were comparable in size and appearance. Table III shows the growth over a period of five days of the metacercariae, each measurement representing the average for ten specimens which had been freed from their cysts. The metacercaria becomes immediately infective upon encystment, but a higher



Figs. 8–14. *Lechriorchis primus, Lechriorchis tygarti* n.sp., and *Caudorchis eurinus* n.gen. et sp., cercaria and metacercaria stages.

Fig. 8. Cercaria of *L. primus*. Stylet and ducts of penetration glands to right at 675 × magnification.
Fig. 9. Cercaria of *L. tygarti*.
Fig. 10. Cercaria of *C. eurinus*.
Fig. 11. Metacercaria of *L. primus* freed from cyst, five days after infection.
Fig. 12. Metacercaria of *L. primus* in cyst, five days after infection.
Fig. 13. Metacercaria of *L. tygarti* freed from cyst, five days after infection.
Fig. 14. Metacercaria of *C. eurinus* freed from cyst, five days after infection.

Table III. *Average measurements of metacercariae when freed from the cyst, intervals of twenty-four hours for period of five days. Ten specimens measured for each interval.*

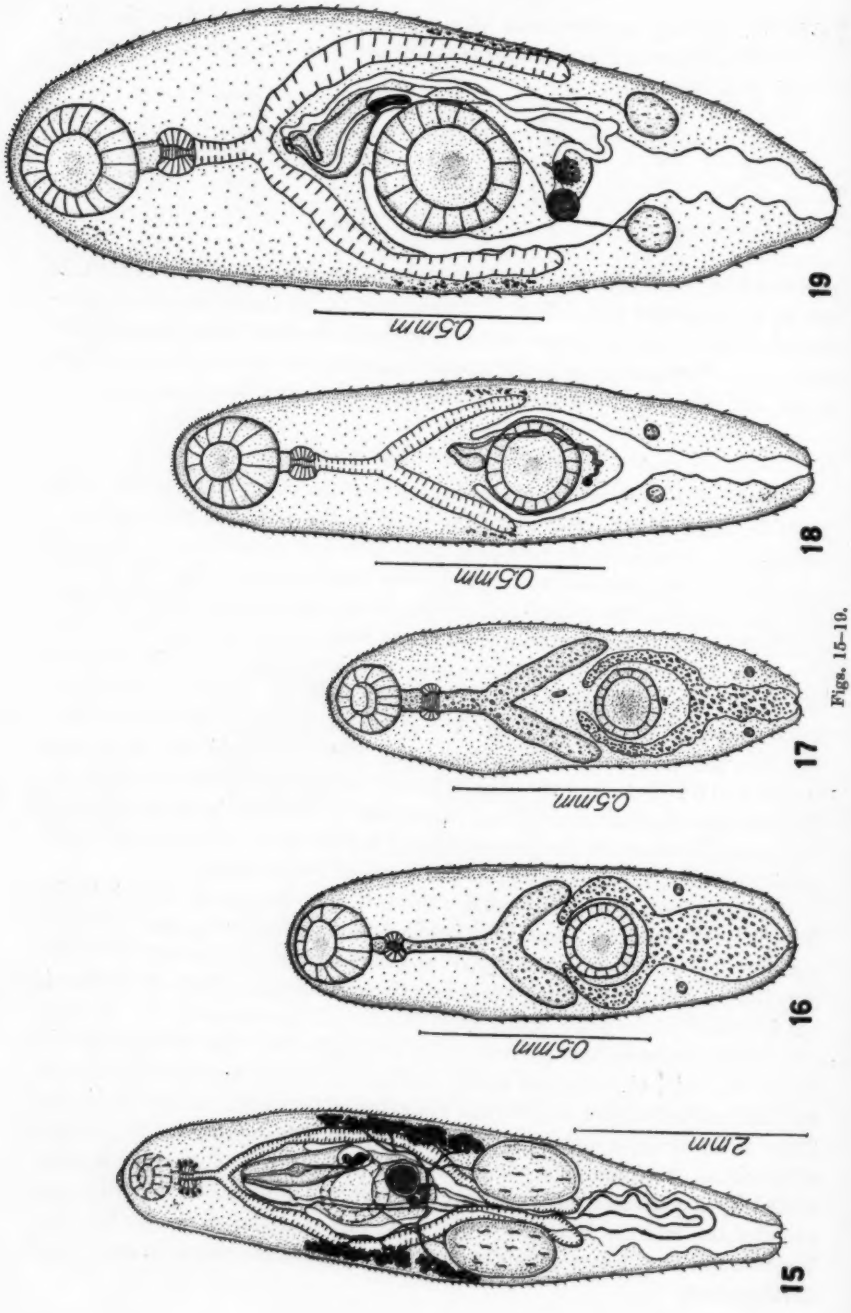
Time in hours	Length in mm.	Width in mm.	Oral sucker in mm.	Ventral sucker in mm.
24	0·57	0·25	0·070	0·075
48	0·66	0·30	0·11	0·11
72	0·91	0·27	0·13	0·11
96	1·00	0·32	0·14	0·13
120	1·01	0·43	0·16	0·13

percentage of infestation was obtained in snakes when older cysts were used. It can be assumed that they excyst in the stomach of the snake host, since metacercariae were found to excyst within thirty minutes after being exposed to a suspension of the scrapings of the stomach mucosa and they would not excyst in bile and extracts of the pancreas, small intestine, and large intestine.

Developmental stages in the definitive host.

After excysting in the stomach of the snake, the young fluke passes to the small intestine where, after attaching itself to the plicae of the mucosa, it remains for a considerable period of time. The first significant development occurs two weeks after the worm reaches the small intestine (Fig. 16). At this stage the anlagen of the testes begin to take form, appearing as two symmetrical germinal centres in the posterior third of the body. There has been practically no growth in the animal as a whole and there is no evidence of feeding. The intestinal caeca have not increased in length and the excretory bladder resembles that of the metacercaria. The worm at this stage measures 1·05 × 0·30 mm. for an average of ten specimens. The oral sucker has an average diameter of 0·16 mm. and the ventral sucker an average diameter of 0·20 mm. The heaviest infestations are always recovered in the small intestine, although the number of worms to become established in this region represent but a small portion of the number of metacercariae ingested by the snake.

In young worms taken from the small intestine, one month after infection (Fig. 17), the intestinal caeca have increased in size and are gorged with food particles. The pharynx is spread apart and the prepharynx is greatly distended. This indicates that the young worm is actively feeding and that an increase in size is about to take place, although at this time it measures but 1 × 0·3 mm., practically the same size as two weeks after infection. The suckers likewise remain the same size, the oral sucker having an average diameter of 0·18 mm. and the ventral sucker an average diameter of 0·21 mm. for ten specimens. There is, however, a development of the reproductive system as the anlagen of the cirrus pouch and ovary have begun to differentiate. The former is represented by a germinal mass midway between the intestinal bifurcation and acetabulum, and the latter by a like mass just posterior to the acetabulum. The two spherical testes remain approximately the same size as in two weeks' development.



Figs. 15–19.

Following the first two months of development in the small intestine, there is a period of approximately five months during which time the worms cease to develop or increase in size, which can probably be correlated with the hibernation period of the snake host.

Then at the end of seven months of residence in the small intestine, the trematode (Fig. 18) leaves the plicae of the intestinal mucosa and starts its migration towards the lung. At this stage of development it is still quite small, ten specimens averaging 1·3 × 0·50 mm. The caeca are somewhat longer, but there is no appreciable increase in size of the suckers. There is, however, considerable development in the reproductive system. The testes now measure 0·02 mm. in diameter and the ovary has assumed a definite shape. The cirrus pouch is formed and the uterus can be seen as a short loop extending from the ovary to the cirrus sac. The vitellaria appear as minute aggregations of follicles lateral to the terminal portion of the caeca. The increase in length has apparently taken place posterior to the testes, as they are now more anterior and the distance between the acetabulum and the posterior end is greater. The passage through the stomach of the snake is completed rather rapidly, as young trematodes were rarely found in the stomach, even of experimentally infected animals.

In contrast to the rapid passage through the stomach, the trematode spends from two to three months migrating the length of the oesophagus. At the end of ten months, most of the worms are found in the first part of the oesophagus and first part of the lung. At this stage of development (Fig. 19), ten specimens measured on an average 1·78 × 0·57 mm. The suckers have now assumed the ratio of the adult worm, the oral sucker having an average diameter of 0·24 mm. and the ventral sucker an average diameter of 0·28 mm. The intestinal caeca have increased in length, now extending posterior to the acetabulum on a level with the ovary. The greatest development, however, has occurred in the reproductive system. The testes have increased to 0·10 mm. in diameter and the vas deferens extends forward to the fully formed cirrus sac. The cirrus sac contains a seminal vesicle, long cirrus and prominent prostate glands. The genital pore is evident and is located close behind the intestinal bifurcation. The ovary is definitely formed and measures 0·05 mm. in diameter. There is a short oviduct although it has grown posteriorly a little. The follicles

Legends to Figs. 15–19.

Figs. 15–19. *Lechriorchis primus*, developmental stages in experimentally infected snake.

Fig. 15. Adult, dorsal view.

Fig. 16. A developmental stage in the small intestine, two weeks after infection, showing differentiation of anlagen of testes.

Fig. 17. A developmental stage in the small intestine, four weeks after infection, showing differentiation of anlagen of the ovary and cirrus pouch.

Fig. 18. A developmental stage in the lower part of oesophagus, seven months after infection, showing differentiation of uterus and vitellaria.

Fig. 19. An almost sexually mature specimen taken from the first part of the oesophagus, eleven months after infection.

of the vitellaria have increased in number, but the vitellarian ducts are not evident. Table IV presents an outline of the size of the young worms and the differentiations of the reproductive system of the four stages of development studies.

Table IV. *Data on the developmental stages of* Lechriorchis primus.

Age of infestation	Length in mm.	Width in mm.	Oral sucker in mm.	Ventral sucker in mm.	Reproductive system
2 weeks	1·05	0·30	0·16	0·20	Testes appear
1 month	1·0	0·30	0·18	0·21	Ovary and cirrus pouch appear
7 months	1·3	0·50	0·18	0·21	Vitellaria and uterus appear
10 months	1·78	0·57	0·24	0·28	Ovarian complex develops

During the first few months of residence in the lung, the trematode increases rapidly in size and approaches sexual maturity. First the uterus begins to fill with eggs and the loop consequently grows posteriorly. The eggs produced the first summer are not fertilised and it is highly probable that copulation does not occur until the next spring when the trematode may be said to become sexually mature.

Just how long the worm continues to live in the lung is merely a matter of conjecture. However, when a great many snakes are examined one is impressed with the extremely small number of trematodes that have reached the maximum size as compared with the number of immature worms found in the small intestine. If the adult worm were to live in the lung for any considerable period of time, heavy infestations should be the rule rather than the exception. It seems very probable that the length of life in the lung does not exceed two years. Frequently adult worms have been found in the large intestine of naturally infected snakes, but these trematodes were usually dead and in many cases only fragments remained. This would seem to indicate that the worms are eliminated from the snake by being swallowed and passed with the faeces after they had probably wandered into the mouth. Therefore, it might be concluded that the life history of *Lechriorchis primus*, from the time it enters the small intestine as a developing worm until it is eliminated, is probably not more than three years.

The life history of *Lechriorchis tygarti*, n.sp.

The second species, of which the life history is to be considered, is also a member of the genus *Lechriorchis*. This trematode was found in the lung of two species of garter snakes, *Thamnophis sauritus* and *T. sirtalis*, collected in the vicinity of Tygart River, Randolph County, West Virginia. In general structure it is easily distinguishable from *Lechriorchis primus* Stafford 1905, the type species of the genus, chiefly in the size of the acetabulum and the length of the intestinal caeca. In many respects it is more closely related to *L. plesientera* Sumwalt, the other American species of this genus. The ratio of the diameter

of the suckers is greater in *L. tygarti* n.sp. and the seminal vesicle is much larger, occupying about one-half of the cirrus pouch. In *L. tygarti* the intestinal caeca extend only to the testes, whereas in *L. plesientera* they extend between the testes and to a considerable distance beyond them. Since this trematode differs from *L. plesientera* in three major respects, I have considered it to be a new species and have assigned to it the name *L. tygarti* after the locality from which it was collected. The following description of *L. tygarti* is based on studies made upon 143 specimens, and all measurements represent the actual measurements of the smallest and largest sexually mature specimens selected from the entire group.

Lechriorchis tygarti n.sp.

(Figs. 20 and 21.)

Specific diagnosis—Lechriorchis. Cuticula beset with small spines, more numerous anteriorly. Body 6·95 × 1·65 mm. in the largest specimen studied and 2·21 × 0·64 mm. in the smallest sexually mature specimen. Oral sucker slightly oval, 0·21–0·52 mm. wide. Acetabulum circular, 0·37–0·86 mm. in diameter, situated 2·45 mm. from anterior end in the larger specimen and 0·68 mm. in the smaller. Prepharynx short. Pharynx strongly muscular, 0·14–0·21 mm. wide. Oesophagus 0·11–0·50 mm. long. Intestinal caeca extend to or almost to anterior margin of testes with ends turned toward the centre of the body. Testes oval, 0·45–0·50 × 0·20–0·35 mm., situated in anterior part of posterior third of body. Cirrus pouch cylindrical, 0·45–1·31 mm. long, extending vertically across and never to centre of acetabulum, containing a large seminal vesicle which occupies approximately one half of the cirrus pouch. Ovary spherical, 0·18–0·27 mm. in diameter, situated to right of midline just posterior to acetabulum. Vitellaria composed of compact follicles, lateral to and slightly extending over intestinal caeca. Eggs oval, operculate, dark brown, 45–53 × 20–34μ.

Hosts: *Thamnophis sauritus, T. sirtalis.*

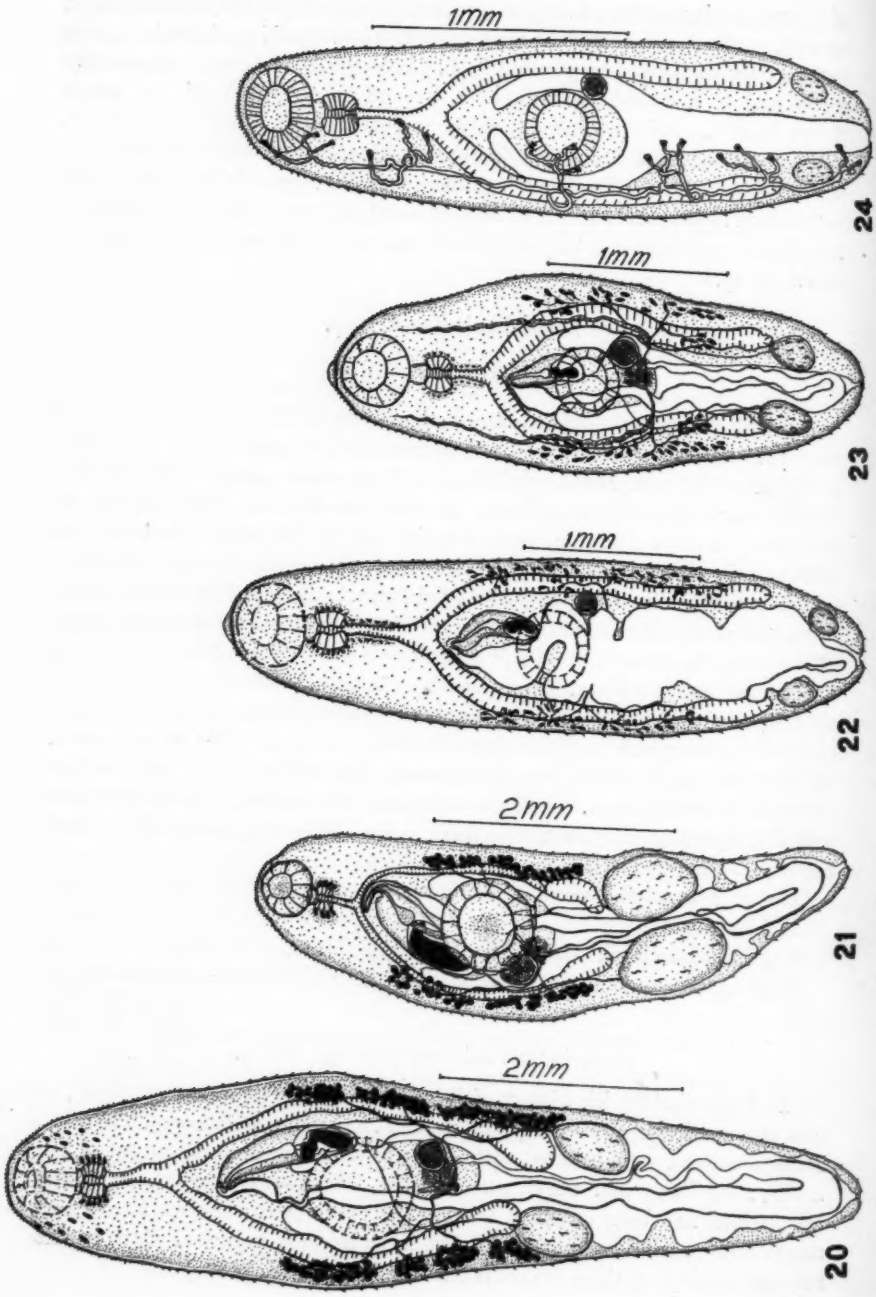
Habitat: Lung.

Locality: Randolph County, West Virginia, U.S.A.

Type specimen: U.S. Nat. Mus. Helm. Coll. No. 8625.

Experimental evidence of the life history.

To determine the life history of *Lechriorchis tygarti* the same procedure, as was pursued in the life history studies of *L. primus*, was followed. Tadpoles, *Rana clamitans*, were infected with a twenty-four-hour cercaria output of five snails, *Physella gyrina*. Seven garter snakes, *Thamnophis sauritus* and *T. sirtalis*, which had been collected ten months previous to the time the experiments were started, were each fed five large infected tadpoles. Data on the autopsy findings of these snakes is outlined in Table V.



Figs. 20–24.

Table V. *An outline of the autopsy records of snakes experimentally infested with* Lechriorchis tygarti *n.sp.*

Host	Age of infestation in months	No. of worms	Position
1. *T. sauritus*	4	30	Small intestine
2. *T. sauritus*	4	15	,,
3. *T. sirtalis*	4	12	,,
4. *T. sirtalis*	5	10	,,
5. *T. sirtalis*	5	13	,,
6. *T. sauritus*	7	34	Oesophagus
7. *T. sauritus*	10	15	First part of oesophagus

Three of the snakes (Hosts 1, 2, 3, Table V) were autopsied at the end of four months' infection and thirty, fifteen, and twelve worms respectively were found in the small intestine. One month later two more snakes (Hosts 4 and 5, Table V) were examined and worms were recovered from the small intestine which were comparable in size and development with those recovered at the end of four months' infection. The fact that the worms were still in the small intestine at the end of five months and were only slightly developed over the metacercaria stage suggested that the development in the definitive host would extend over a relatively long period much like that described for *Lechriorchis primus*. Therefore, the next snake (Host 6, Table V) was not examined until seven months after the initial infection, since at the end of this period it was expected that worms would be migrating from the small intestine. This assumption was correct for thirty-four young worms were recovered from the lower oesophagus and not a single worm was found in the small intestine. The remaining snake (Host 7, Table V) was not examined until ten months after the initial infection and three months after the time the worms should have entered the oesophagus. When this final snake was autopsied, fifteen worms were recovered from the first part of the oesophagus which were not quite sexually mature, but which were sufficiently developed to make an identification possible. The presence of a relatively large acetabulum, prominent seminal vesicle and short intestinal caeca, all specific characters of *L. tygarti*, proved beyond reasonable doubt that the cercaria used in these experiments was the cercaria stage of *L. tygarti*.

In order to obtain further proof for the identity of this cercaria, laboratory-bred snails, *Physella gyrina*, were infected with the ova of *L. tygarti*. Five

Legends to Figs. 20–24.

Figs. 20–24. *Lechriorchis tygarti* n.sp. and *Caudorchis eurinus* n.gen. et sp., adult specimens.

Fig. 20. *L. tygarti*, dorsal view, maximum maturity, showing short intestinal caeca, large seminal vesicle, and large acetabulum.

Fig. 21. *L. tygarti*, medium sized specimen, ventral view.

Fig. 22. *C. eurinus*, dorsal view, maximum maturity, showing scattered vitellaria, long intestinal caeca, and posterior testes.

Fig. 23. *C. eurinus*, medium sized specimen, dorsal view, showing main collecting ducts of excretory bladder full of dark excretory concretions.

Fig. 24. *C. eurinus* showing excretory system, drawing made from living specimen.

weeks later these snails gave off cercariae which were found to be morphologically identical with the cercariae used in the foregoing experiment as they agreed exactly in size and possessed the same characteristically large acetabulum.

The morphology of the life history stages of *Lechriorchis tygarti* is fundamentally the same as that described for *L. primus*. The egg is smaller, 43–53 × 20–34μ, but it is of the same general shape and character. The sporocyst stages have the same simplicity of structure and occupy the same position in the snail host. It is possible, however, to distinguish between the cercariae of the two forms, since the cercaria of *L. tygarti* (Fig. 9) is larger and the size ratio of the suckers is greater. The metacercaria (Fig. 13) is also larger, but it would be difficult to distinguish it from that of *L. primus* unless, perhaps, comparisons were made between specimens of the two forms which were known to be of the same age of infection.

The developmental stages of *L. tygarti* in the snake host are practically identical with those described for *L. primus*. There is the same gradual differentiation of the reproductive system and the same relatively long period of residence in the alimentary canal. Therefore, the life history of *L. tygarti* closely parallels that of *L. primus* both in time required to complete the life cycle as well as the species of hosts parasitised.

The life history of *Caudorchis eurinus* n.gen. et sp.

The life history of the third species, *Caudorchis eurinus* n.gen. et sp., is also similar to that of *Lechriorchis primus*. Not only does it occur in the same species of snake hosts collected from the same region, but it also has larval stages in the same species of intermediate hosts which are almost identical with these stages of *L. primus*. The adult stage, however, possesses certain characteristics which exclude it from the genus *Lechriorchis*. Since it cannot be placed in this genus, to which it undoubtedly is more closely related than any of the other genera of the subfamily Reniferinae, it is considered to belong to a new genus and species for which the name *Caudorchis eurinus* is proposed.

There are four distinct morphological differences which form a logical basis for considering the genus *Caudorchis* distinct from *Lechriorchis*. The suckers of *Caudorchis* are approximately equal in size, whereas in the genus *Lechriorchis*, the ventral sucker is always larger than the oral. In *Caudorchis eurinus*, the intestinal caeca are lateral in position and longer, in proportion to the body length than *Lechriorchis* spp. in which the intestinal caeca tend to be or are clasped between the testes. Also the vitellaria of *Caudorchis eurinus* are small and scattered as compared with the rather large compact vitellarian follicles of *Lechriorchis primus* and other species of this genus. The most striking point of difference is in the position and relation of the testes to the intestinal caeca. In *Caudorchis eurinus* the testes are located in the extreme posterior end of the body and the ends of the intestinal caeca almost impinge upon them, while in *Lechriorchis* spp. the intestinal caeca are clasped or tend to be clasped

between the testes. The generic and specific diagnosis of this new trematode is as follows:

Genus **Caudorchis** n.gen.

Generic diagnosis—Reniferinae. Body elliptical, spinous. Suckers approximately equal in size. Pharynx strongly muscular. Intestinal caeca narrow, extending laterally into posterior one-third of body. Genital pore close behind intestinal bifurcation. Testes small, opposite or oblique, in posterior end of body. Vitellaria composed of diffuse follicles situated laterally and ventrally to intestinal caeca and extending from level of genital pore to near termination of intestinal caeca. Uterus single loop extending into extreme posterior end of body between testes, uncoiled. Eggs numerous, ovoid, operculate and light brown.

Parasitic in lung of garter snakes.

Type species *Caudorchis eurinus* n.sp.

Caudorchis eurinus n.sp.

(Figs. 22–24.)

Specific diagnosis—Caudorchis. Body elliptical, dorso-ventrally flattened, anterior end rounded, tapering to blunt point posteriorly. Cuticular spines present, more numerous anteriorly. Body 1·94–3·27 × 0·58–0·78 mm. in the region of the acetabulum. Oral sucker subterminal, circular, 0·29–0·38 mm. in diameter. Acetabulum spherical, 0·29–0·38 mm. in diameter, in the centre of the body 0·70–1·24 mm. from the posterior margin of the oral sucker. Prepharynx 0·046–0·077 mm. long. Pharynx strongly muscular 0·15–0·18 mm. wide. Pharyngeal glands present. Oesophagus short and slender, 0·15–0·23 mm. long. Intestinal caeca narrow extending to the posterior testes. Anterior and posterior main collecting ducts of the excretory system full of dark excretory concretions. Testes oval, 0·15–0·26 × 0·12–0·18 mm., situated near posterior end of body, arranged opposite or obliquely. Cirrus pouch cylindrical, 0·27–0·37 mm. long, extending vertically across but never beyond centre of acetabulum. Ovary spherical, 0·10–0·14 mm. in diameter, situated to the left of the median line just posterior to the acetabulum. Vitellaria composed of small scattered follicles, two groups anteriorly and two groups posteriorly; anterior groups extending to on a level with the genital pore, the posterior groups to within a short distance of the termination of the intestinal caeca. Uterus a descending and an ascending loop extending between the testes to the posterior end of the body and terminating in a moderately well-developed metraterm. Metraterm extends along right side of the cirrus pouch and opens into genital sinus on a level with the male aperture. Eggs oval, light brown, 42–46 × 18–23μ.

Host: *Thamnophis sauritus, T. sirtalis.*

Habitat: Lung.

Locality: Cheboygan County, Michigan, U.S.A.

Type specimen: U.S. Nat. Mus. Helm. Coll. No. 8630.

The foregoing description of *Caudorchis eurinus* is based upon studies made on twenty specimens comprising both young sexually mature worms and worms which had attained the maximum size. All measurements stated in connection with the description represent the range between the smallest sexually mature specimen and the largest specimen of this group.

In addition to the characters mentioned in the specific diagnosis, it was possible to work out the complete excretory system of the adult. The excretory tubules of *C. eurinus* are filled with dark excretory concretions which very sharply outline the limits of the tubules. Since the body parenchyma of this form is relatively clear, the individual flame cells could be located with ease which could not be done with either of the two species of *Lechriorchis* studied in which the parenchyma is much more dense. The distribution and number of flame cells (Fig. 24) in *Caudorchis eurinus* is identical with this system in its cercaria stage which was found to be of the "2 × 6 × 3" type. The flame cell pattern has been determined for the adult stage of at least one other species of the Reniferinae. McCoy (1928), from a study of living material, found the flame cell pattern of *Dasymetra conferta* Nicoll also to be of the "2 × 6 × 3" type.

Experimental evidence of the life history.

The first information on the life history of *Caudorchis eurinus* was obtained from a series of preliminary studies with a stylet cercaria similar to the cercaria of *Lechriorchis primus* but which could be distinguished from it on the basis of the length of the intestinal caeca. The fact that in *Caudorchis eurinus* the intestinal caeca are likewise long suggested the possibility that this cercaria was the larval stage of that species. Consequently tadpoles were infected with the cercaria, and after a period of five days were fed to garter snakes. Several months later the snakes were examined and numerous developing worms were found which had developed sufficiently to suggest the structure of *C. eurinus*. After the preliminary studies, which indicated the general course of the life cycle of *C. eurinus*, were completed, a series of experiments were started which made possible a detailed study of the life history stages. Snails, *Physella gyrina*, were infected with the ova of *Caudorchis eurinus* and within the course of five weeks cercariae were obtained which were identical to the cercariae used in the preliminary experiments.

Tadpoles, *Rana clamitans*, were then infected with the experimental cercariae and fed to thirteen snakes (Table VI) consisting of two species of garter snakes, *Thamnophis sauritus*, *T. sirtalis*, and one species of water snake, *Natrix septemvittata*. These snakes were autopsied according to the schedule outlined in Table VI and the alimentary canal carefully examined for developing worms.

The four water snakes (Hosts 2, 3, 4, and 5, Table VI) were found to be negative, which suggests that *Natrix septemvittata* will not serve as a host for this parasite, but all the garter snakes were experimentally infected. The first

garter snake (Host 1, Table VI) was autopsied at the end of two months' infection and 243 worms were recovered from the small intestine. Two months later, two more snakes (Hosts 8, 9, Table VI) were autopsied and forty-five and ten worms respectively were recovered from the small intestine which were slightly larger than those recovered at the end of two months' infection. The most conclusive information concerning the migration of the young worms was obtained from the examination of Hosts 10 and 11, Table VI. In these two

Table VI. *Outline of results from snake hosts experimentally infected with* Caudorchis eurinus.

Host	Age of infestation in months	No. of worms recovered	Organ parasitised
1. *T. sirtalis*	2	254	Small intestine
2. *N. septemvittata*	2	—	—
3. *N. septemvittata*	2	—	—
4. *N. septemvittata*	3	—	—
5. *N. septemvittata*	3	—	—
6. *T. sirtalis*	3	86	Small intestine
7. *T. sauritus*	3	164	"
8. *T. sauritus*	4	45	"
9. *T. sirtalis*	5	10	"
10. *T. sauritus*	6	56	Small intestine, stomach, and lower oesophagus
11. *T. sauritus*	7	60	Stomach and lower oesophagus
12. *T. sauritus*	10	34	Upper oesophagus
13. *T. sirtalis*	11	22	"

snakes, the worms were found in the stomach and lower part of the oesophagus which indicated that the migration to the lung begins from six to seven months after infection. In worms of this age there is a considerable degree of differentiation of the reproductive system, the major reproductive organs having been formed, namely, the testes, ovary, and cirrus pouch. The two remaining snakes (Hosts 12, 13, Table VI) were not examined until at the end of ten months' infection. Practically sexually mature worms were recovered from the first part of the oesophagus, which were unmistakably identical with the adult worms found in the lung and from which ova had been used to infect snails.

There can be little doubt, then, that the foregoing experiments definitely indicate the life history of *Caudorchis eurinus*. The fact that a 100 per cent. infestation was experimentally established in garter snakes, and further, the finding of three definite stages of development in the definitive host, would strongly support this conclusion. Also it appears that very definite proof for the life history was obtained in Hosts 11 and 12 (Table VI) in which almost sexually mature worms were recovered from the first part of the oesophagus. Therefore, the life history of *C. eurinus* is essentially the same as that described for *Lechriorchis primus* both in time required for completion as well as the species of snakes, snails and tadpoles included in the hosts parasitised.

Life history stages of Caudorchis eurinus.

To give a detailed description of the life history stages of *C. eurinus* would be a matter of repetition, since they agree in the main with what has already been said concerning these stages of *Lechriorchis primus*. There are, however, certain points of difference which will be discussed. The eggs are slightly smaller and are a little lighter in colour than those of *L. primus* or *L. tygarti*. In general structure, the sporocyst stages appear to be identical with those of *L. primus* and they occupy the same position in the snail host. Superficially the two cercariae are indistinguishable as they had exactly the same body size and there is no difference in the size and position of the suckers.

In the cercaria of *Caudorchis eurinus*, however (Fig. 10), the intestinal caeca extend beyond the acetabulum into the posterior third of the body as compared with the rather short intestinal caeca of the cercaria of *Lechriorchis primus* (Fig. 8). There also appears to be a slight difference in the character of the excretory bladder. The arms of the excretory bladder of the cercaria of *Caudorchis eurinus* do not as completely encircle the acetabulum nor does the stem of the excretory bladder as nearly approach the posterior margin of the acetabulum. In the metacercaria stage (Figs. 11 and 14) these differences are even more evident, because the cystogenous glands are exhausted and the limits of the intestinal caeca, as well as the outline of the excretory bladder, are more readily seen.

The developmental stages in the definitive host have already been referred to in a previous discussion. However, it is worth while to call attention to the similarities and differences between these stages and those described for *Lechriorchis primus*. It is an interesting fact that in the case of *Caudorchis eurinus*, an entirely different genus, practically the same time is required to complete the developmental stages in the alimentary canal as was observed for the two species of *Lechriorchis*. There are also the same apparent three stages of differentiation of the reproductive organs corresponding to the three periods of residence in the small intestine, lower oesophagus, and first part of the oesophagus. It is possible, however, to distinguish the young worms of *Caudorchis eurinus* in any stage of development because the position of certain organs is distinctly different from that in *Lechriorchis primus* or *L. tygarti*.

Even in the small intestine, during which time the anlagen of the testes take a definite shape, it is easy to distinguish this form because the testes are always more posterior. Also the intestinal caeca of *L. primus* and *L. tygarti* are very short at this time, while in *Caudorchis eurinus* they are long and extend into the posterior end of the body. In *C. eurinus* the ovary, cirrus pouch, and uterus have become differentiated when the young worms reach the lower part of the oesophagus which is similar to *Lechriorchis tygarti* and *L. primus*. At this stage the suckers have assumed the size ratio of the adult, which also affords a means of distinguishing this form from either *L. primus* or *L. tygarti*.

When the young worms reach the first part of the oesophagus, the entire

reproductive system is formed, consisting of the testes, ovary, vitellaria, Mehlis glands, Laurer's canal, and cirrus pouch with a definite seminal vesicle, cirrus and prostate glands. No ova have as yet been formed and there is no indication that the worms are ready for copulation. Probably a period of further growth is required in the lung before eggs are produced. One would conclude, then, that practically the same time is required to complete the full life cycle of *Caudorchis eurinus*, from the time viable eggs are ingested by the snail host until an infestation is established in the lung of the definitive host producing ova, as was observed for *Lechriorchis primus* and *L. tygarti*.

GENERAL DISCUSSION.

With the completion of the present studies, we now have information on the life histories of eight species belonging to seven genera of the subfamily Reniferinae. All of these species show a remarkable uniformity in the course of their life cycles and in the structure of their larval stages, a fact which indicates the importance of these characters in any adequate definition of this subfamily.

In connection with the present investigations, the miracidia of six species of the subfamily Reniferinae were studied, two of the genus *Lechriorchis* and one each of the genera *Caudorchis*, *Renifer*, *Pneumatophilus*, and *Macrodera* (= *Saphedra*). In each case the miracidia were so similar that no structural differences could be found whereby they could be distinguished. In a species of *Renifer*, however, the ova were found to hatch in the open rather than in snail's stomach as is the case with the species of the other genera.

At present we have adequate information on the cercaria stage of seven species of six genera belonging to the Reniferinae. In all of these the structure of the cercaria shows a striking similarity. One of the most important characteristics is the Y-shaped excretory bladder with the lateral arms almost encircling the acetabulum. This type of excretory bladder was first described by Cort (1914, 1915) in *Cercaria brevicaeca* and was at that time unique. All of the cercariae described for the Reniferinae, with the exception of those of *Dasymetra conferta* and *Caudorchis eurinus* which have much longer intestinal caeca than the others, are so similar to *C. brevicaeca* that they can be distinguished from it and from each other only with the greatest difficulty. Not only are all of these cercariae similar in structure, but they also all develop in species of *Physella* and, in those cases where further development is known, penetrate and encyst in tadpoles.

The structure of the larval stages of digenetic trematodes has been used, in certain cases, as a basis for determining the doubtful genetic relationships of the adult forms. Cort (1917) was the first to point out the importance of the excretory system of the cercaria stage as a basis for the establishment of a natural system of classification. La Rue (1926), in a study of the Strigeidae, grouped this family with the Schistosomatoidea in a single order Strigeatoidea on the basis of the similarity in structure of the larval stages. The life history

studies of the Reniferinae support the conclusions of these investigators that the characteristics of the larval stages of digenetic trematodes are an important aid in developing a natural classification.

It is proposed, therefore, that larval characters, especially those of the miracidium and cercaria be used as diagnostic for the subfamily Reniferinae. In the miracidia the small number of germ cells and the two huge penetration glands appear to be typical. As previously noted, the structure of the cercariae is remarkably uniform with a small stylet and a characteristic Y-shaped bladder. Also the same species of snail and tadpole hosts are parasitised by these stages. With these larval characters known for the species of practically all the genera of this subfamily, it is now possible to establish a logical basis for the classification of the Reniferinae as a natural group.

Abstract summary.

1. The life histories of *Lechriorchis primus* Stafford, *L. tygarti* n.sp. and *Caudorchis eurinus* n.gen. et sp. have been experimentally completed in three hosts, the first complete life histories to be worked out for species of the subfamily Reniferinae.

2. The definitive hosts of the three forms were found to be two species of garter snakes, *Thamnophis sauritus* and *T. sirtalis*.

3. Three species of snails, *Physella gyrina*, *P. parkeri*, and *P. ancillaria*, have been found to serve as the first intermediate host in the life cycles of *Lechriorchis primus* and *Caudorchis eurinus* n.gen. et sp., and two species of snails, *Physella gyrina* and *P. heterostropha*, in the life cycle of *Lechriorchis tygarti* n.sp.

4. The tadpoles of two species of frogs, *Rana clamitans* and *R. pipiens*, were found to serve as the second intermediate hosts in the life cycles of all three trematodes. The cercariae penetrate larvae of *Triturus* and small fish, but live only a short time in these animals.

5. Every stage in the life history of *Lechriorchis primus*, including egg, miracidium, mother sporocyst, daughter sporocyst, cercaria, metacercaria, and developmental stages in the definitive host, has been described in detail.

6. The mother sporocyst of forms having a stylet cercaria is described for the first time.

7. The flame cell pattern of the cercariae of *L. primus*, *L. tygarti* n.sp., and *Caudorchis eurinus* n.gen. et sp. has been determined to be of the "$2 \times 6 \times 3$" type. Also the adult stage of *C. eurinus* was determined to have the same type.

8. It has been pointed out that the life histories of the members of the subfamily are uniform in that their life history stages display a remarkable similarity.

9. It has been suggested that this uniform type of life cycle and remarkable similarity of larval stages offer the most logical basis for establishing the subfamily Reniferinae as a natural group.

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CORRIGENDUM.

Page 186, line 2, for *rhodesiense* read *gambiense*.

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